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(54) Title: **PROCESS FOR THE PREPARATION OF RESIN-BOUND CYCLIC PEPTIDES**

(57) Abstract

This invention is directed to a process for the solid phase, fragment-based synthesis of resin-bound cyclic peptide analogs of parathyroid hormones and analogs of parathyroid hormone-related proteins, which analogs contain at least one bridge between the side chains of two non-adjacent amino acid residues, and to peptide fragments useful therefor.

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## PROCESS FOR THE PREPARATION OF RESIN-BOUND CYCLIC PEPTIDES

## FIELD OF THE INVENTION

5 This invention is directed to a process for the fragment-based preparation of resin-bound cyclic peptide analogs of parathyroid hormones and analogs of parathyroid hormone-related proteins, which analogs contain at least one bridge between the side chains of two non-adjacent amino acid residues. More particularly, the invention is directed to a process for the solid-phase synthesis of such cyclic peptide analogs, and to peptide fragments useful therefor.

10

## BACKGROUND OF THE INVENTION

Cyclic peptide subunits are present in a wide variety of peptides possessing useful biological activity, including parathyroid hormone analogs and parathyroid hormone-related protein analogs, 15 vasoactive peptide analogs, cholecystokinin analogs, tumor necrosis factor (TNF) derived peptides, calcitonin analogs, somatostatin analogs, cell adhesion modulators, growth hormone releasing factor (GRF) analogs, bradykinin antagonists, analogs of tyrosine activation motifs (TAM Mimics) and amylin agonists. Of particular interest are the cyclic peptide parathyroid hormone (hPTH) analogs and parathyroid hormone-related protein (hPTHrP) analogs.

20 Human parathyroid hormone (hPTH) is an 84 amino acid protein which is a major regulator of calcium homeostasis. Parathyroid hormone-related protein (hPTHrP) is a 139 to 171 amino acid protein with N-terminal homology to hPTH. The N-terminal fragments of hPTH and hPTHrP, particularly those consisting of amino acids 1-34, retain the full biological activity of the parent hormone.

hPTH(1-34) has the following amino acid sequence:

25 Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys-His-Leu-Asn-Ser-Met-  
Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu-Gln-Asp-Val-His-Asn-Phe.

hPTHrP(1-34) has the following amino acid sequence:

Ala-Val-Ser-Glu-His-Gln-Leu-Leu-His-Asp-Lys-Gly-Lys-Ser-Ile-Gln-Asp-Leu-  
Arg-Arg-Arg-Phe-Phe-Leu-His-His-Leu-Ile-Ala-Glu-Ile-His-Thr-Ala.

30 The biological activity of hPTH is reflected in the activation of two secondary messenger systems: G-protein coupled adenylyl cyclase (AC) and G-protein coupled and uncoupled protein kinase C (PKC) activity. The N-terminal fragments hPTH(1-34)OH and hPTH(1-31)NH<sub>2</sub> have been demonstrated to be anabolic with respect to bone formation in humans and ovariectomized rats, respectively. This increase in bone growth has been demonstrated to be coupled with stimulation of

adenylyl cyclase activity. Analogs of these *N*-terminal fragments have significant therapeutic potential for the treatment of physiological conditions associated with bone cell calcium regulation including hypocalcemia; osteoporosis; osteopenia; and disorders associated with osteoporosis and osteopenia such as hyperparathyroidism, hypoparathyroidism, and Cushing's syndrome; glucocorticoid- and immunosuppressant-induced osteopenia; and bone fracture and bone refracture repair.

It has also been established that deletion of up to six amino acid residues from the *N*-terminus of hPTH(1-34) markedly decreases the resulting analog's ability to stimulate adenylyl cyclase while having little effect on receptor binding. Thus, analogs of hPTH(1-34) truncated by up to six amino acid residues at the *N*-terminus inhibit the action of PTH and are useful in the treatment of disorders characterized by an excess of PTH such as hyperparathyroidism and hyperparathyroidism-related hypercalcemia crisis, hypercalcemia of malignancy, renal failure and hypertension.

Acyclic analogs of hPTH(1-27) to (1-34) are disclosed in United States Patent 4,086,196. Acyclic analogs of hPTH(1-34) and hPTHrP (1-34) are disclosed in U.S. Pat. No. 5,589,452. [Nle<sup>8</sup>, Nle<sup>18</sup>, Tyr<sup>34</sup>, or Phe<sup>34</sup>]hPTH(1-34) are disclosed in U.S. Pat. No. 4,656,250. [Nle<sup>8</sup>, Nle<sup>18</sup>, Tyr<sup>34</sup>]hPTH(1-34) and *N*-truncated derivatives thereof are disclosed in U.S. Pat. Nos. 4,771,124 and 4,423,037. Other acyclic analogs of PTH(1-34) are disclosed in U.S. Pat. Nos. 5,723,577 and 5,434,246, WO 97/02834, EPA 561 412-A1, EPA 747 817-A2, WO-94/02510, WO9603437, and WO9511988-A1. Analogs of hPTH(1-28)NH<sub>2</sub> to hPTH(1-31)NH<sub>2</sub> and [Leu<sup>27</sup>]hPTH(1-28)NH<sub>2</sub> to [Leu<sup>27</sup>]hPTH(1-33)NH<sub>2</sub> are described in U.S. Pat. No. 5,556,940. Acyclic antagonists of the PTH receptor including *N*-terminally-truncated analogs of PTH are disclosed in U.S. Pat. Nos. 5,446,130, 5,229,489, 4,771,124 and 4,423,037.

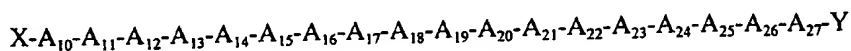
Cyclic and bicyclic analogs of hPTH and hPTHrP have been disclosed. Cyclo(Lys<sup>26</sup>-Asp<sup>30</sup>)[Leu<sup>27</sup>]hPTH(1-34)NH<sub>2</sub> and cyclo(Lys<sup>27</sup>-Asp<sup>30</sup>)hPTH(1-34)NH<sub>2</sub> are disclosed in U.S. Pat. No. 5,556,940. Cyclo(Lys<sup>26</sup>-Asp<sup>30</sup>)[Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>, cyclo(Glu<sup>22</sup>-Lys<sup>26</sup>)[Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>, and cyclo(Lys<sup>27</sup>-Asp<sup>30</sup>)hPTH(1-31)NH<sub>2</sub> are described by Barbier, et al., *J. Med. Chem.* **1997**, *40*, 1373.

Monocyclic and bicyclic derivatives of hPTH(1-34) or hPTHrP(1-34) are disclosed in patent documents WO 96/40193, DE19508672-A1, and by A. Bisello, et al., in *Biochemistry* **1997**, *36*, 3293. Cyclo(Lys<sup>13</sup>-Asp<sup>17</sup>)hPTHrP(7-34)NH<sub>2</sub>, a potent antagonist of the PTH receptor, is disclosed by M. Chorev, et al.,

*Biochemistry* **1991**, *30*, 5698. Also, Kanmera, et al., has described a series of amide-containing analogs of hPTHrP, *Peptide Chemistry* 1993: Okada, Y., ed.; Protein Research Foundation, Osaka, **1994**, 321-

324."

WO 98/51324 discloses cyclic peptide compounds of formula I



and pharmaceutically acceptable salts and prodrugs thereof wherein

X is selected from the group consisting of

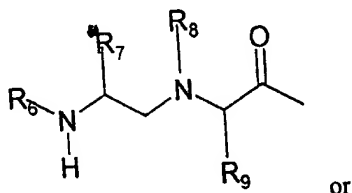
- (a)  $R_{1a}-A_0-A_1-A_2-A_3-A_4-A_5-A_6-A_7-A_8-A_9-$ ,
- 5 (b)  $R_{1a}-A_2-A_3-A_4-A_5-A_6-A_7-A_8-A_9-$ ,
- (c)  $R_{1b}-A_3-A_4-A_5-A_6-A_7-A_8-A_9-$ ,
- (d)  $R_{1a}-A_4-A_5-A_6-A_7-A_8-A_9-$ ,
- (e)  $R_{1a}-A_5-A_6-A_7-A_8-A_9-$ ,
- (f)  $R_{1a}-A_6-A_7-A_8-A_9-$ ,
- 10 (g)  $R_{1a}-A_7-A_8-A_9-$ ,
- (h)  $R_{1a}-A_8-A_9-$ ,
- (i)  $R_{1a}-A_9-$ , and
- (j)  $R_{1a}-$ ;

15 Y is selected from the group consisting of

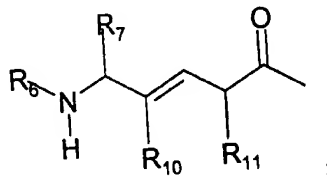
- (a)  $-R_3$ ,
- (b)  $-A_{28}-R_3$ ,
- (c)  $-A_{28}-A_{29}-R_3$ ,
- (d)  $-A_{28}-A_{29}-A_{30}-R_3$ ,
- 20 (e)  $-A_{28}-A_{29}-A_{30}-A_{31}-R_3$ ,
- (f)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-R_3$ ,
- (g)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-A_{33}-R_3$ , and
- (h)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-A_{33}-A_{34}-R_3$ ;

25  $R_{1a}$  is H, alkyl, aralkyl or  $-COR_2$ ;

$R_{1b}$  is  $R_{1a}$  or a group of formula



or



$R_2$  is alkyl, alkenyl, alkynyl, aryl or aralkyl;

$R_3$  is a group of formula  $A_{35}-OR_4$  or  $A_{35}-NR_4R_5$ ;

5  $R_4$  and  $R_5$  are independently H or lower alkyl;

$R_6$  and  $R_9$  are independently H or alkyl;

$R_7$  is alkyl;

10

$R_8$  is H, alkyl or  $COR_2$ ;

$R_{10}$  is H or halogen;

15

$R_{11}$  is alkyl or aralkyl;

$m$  is 1, 2 or 3;

$n$  is 3 or 4;

20

$A_0$  is absent or a peptide of from one to six amino acid residues;

$A_1$  is Ser, Ala, Gly or D-Pro, or an equivalent amino acid thereof;

25

$A_2$  is Ala, Val or Gly, or an equivalent amino acid thereof;

---

$A_3$  is Ala, Ser, Gly or D-Pro, or an equivalent amino acid thereof;

$A_4$  is Glu, Ala or Gly, or an equivalent amino acid thereof;

30

$A_5$  is Ile, His, Ala or Gly, or an equivalent amino acid thereof;

$A_6$  is Ala, Gln, Gly or D-Pro, or an equivalent amino acid thereof;

$A_7$  is Ala, Leu, Gly, or an equivalent amino acid thereof;

$A_8$  is Leu, Nle, Gly or D-Pro, or an equivalent amino acid thereof;

5  $A_9$  is His, Ala, D-Pro or Gly, or an equivalent amino acid thereof;

$A_{10}$  is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, D-Pro,  
-NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

10  $A_{11}$  is Ala, Gly, Leu or Lys, or an equivalent amino acid thereof;

$A_{12}$  is Ala or Gly, or an equivalent amino acid thereof;

15  $A_{13}$  is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or  
-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

$A_{14}$  is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, His, Lys, Orn, Ser, Thr, D-Pro,  
-NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

20  $A_{15}$  is Ala, Gly, Ile, D-Pro or Leu, or an equivalent amino acid thereof;

$A_{16}$  is Asn, Ala, Gly, D-Pro or Gln, or an equivalent amino acid thereof;

25  $A_{17}$  is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, D-Pro,  
-NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

---

$A_{18}$  is Asp, Cys, homo-Cys, Glu, His, Leu, Lys, Orn, Nle, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or  
-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

30  $A_{19}$  is Arg or Glu, or an equivalent amino acid thereof;

$A_{20}$  is Arg or an equivalent amino acid thereof;

$A_{21}$  is Arg, Asp, Cys, homo-Cys, Glu, Lys, Orn, Ser, Thr, Val, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or

-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>22</sub> is Asp, Cys, homo-Cys, Glu, His, Lys, Orn, Phe, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or

-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

5

A<sub>23</sub> is Leu, Phe or Trp, or an equivalent amino acid thereof;

A<sub>24</sub> is Leu or an equivalent amino acid thereof;

10

A<sub>25</sub> is Arg, Asp, Cys, homo-Cys, Glu, His, Lys, Orn, D-Pro, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or

-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>26</sub> is Asp, Cys, homo-Cys, Glu, His, Lys, Orn, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or

-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

15

A<sub>27</sub> is Leu or Lys, or an equivalent amino acid thereof;

A<sub>28</sub> is Ile or Leu, or an equivalent amino acid thereof;

20

A<sub>29</sub> is Ala, Asp, Cys, homo-Cys, Glu, Gln, Lys, Orn, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or

-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>30</sub> is Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or

-NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

25

A<sub>31</sub> is Ile, Leu or Val, or an equivalent amino acid thereof;

A<sub>32</sub> is His, or an equivalent amino acid thereof;

30

A<sub>33</sub> is Asn or Thr, or an equivalent amino acid thereof; and

A<sub>34</sub> is Ala or Phe, or an equivalent amino acid thereof;

A<sub>35</sub> is absent or a peptide of from 1 to 4 amino acids; and

- the side chains of at least one of the following pairs of amino acid residues, A<sub>10</sub> and A<sub>14</sub>, A<sub>13</sub> and A<sub>17</sub>, A<sub>14</sub> and A<sub>18</sub>, A<sub>17</sub> and A<sub>21</sub>, A<sub>18</sub> and A<sub>22</sub>, A<sub>21</sub> and A<sub>25</sub>, A<sub>25</sub> and A<sub>29</sub> and A<sub>26</sub> and A<sub>30</sub> are linked through an amide, ester, disulfide or lanthionine bond to form a bridge, and the side chain of each of the following amino acid residues, A<sub>10</sub>, A<sub>13</sub>, A<sub>14</sub>, A<sub>17</sub>, A<sub>18</sub>, A<sub>21</sub>, A<sub>22</sub>, A<sub>25</sub>, A<sub>26</sub>, A<sub>29</sub>, and A<sub>30</sub>, contributes, at most, to the formation of a single bridge; provided that when the side chains of the following pairs of amino acid residues, A<sub>13</sub> and A<sub>17</sub> or A<sub>26</sub> and A<sub>30</sub> are linked through an amide, disulfide or lanthionine bond to form a bridge, then the side chains of at least one of the following pairs of amino acid residues, A<sub>10</sub> and A<sub>14</sub>, A<sub>14</sub> and A<sub>18</sub>, A<sub>17</sub> and A<sub>21</sub>, A<sub>18</sub> and A<sub>22</sub>, A<sub>21</sub> and A<sub>25</sub> and A<sub>25</sub> and A<sub>29</sub> are also linked through an amide, ester, disulfide or lanthionine bond.

A preferred subset of the peptide compounds of formula I comprises the peptide compounds wherein

- 15 X is selected from the group consisting of
- (a) R<sub>1a</sub>-A<sub>0</sub>-A<sub>1</sub>-A<sub>2</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (b) R<sub>1a</sub>-A<sub>2</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (c) R<sub>1b</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (d) R<sub>1a</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - 20 (e) R<sub>1a</sub>-A<sub>5</sub>-A<sub>6</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (f) R<sub>1a</sub>-A<sub>6</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (g) R<sub>1a</sub>-A<sub>7</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (h) R<sub>1a</sub>-A<sub>8</sub>-A<sub>9</sub><sup>-</sup>,
  - (i) R<sub>1a</sub>-A<sub>9</sub><sup>-</sup>, and
  - 25 (j) R<sub>1a</sub><sup>-</sup>;

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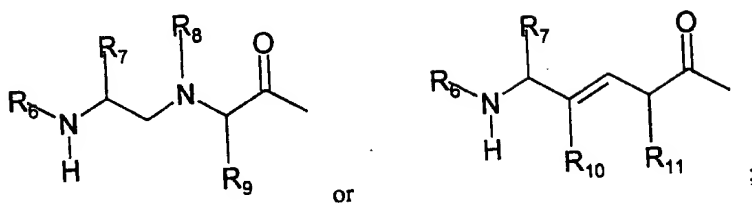
Y is selected from the group consisting of

- (a) -R<sub>3</sub>,
- (b) -A<sub>28</sub>-R<sub>3</sub>,
- 30 (c) -A<sub>28</sub>-A<sub>29</sub>-R<sub>3</sub>,
- (d) -A<sub>28</sub>-A<sub>29</sub>-A<sub>30</sub>-R<sub>3</sub>,
- (e) -A<sub>28</sub>-A<sub>29</sub>-A<sub>30</sub>-A<sub>31</sub>-R<sub>3</sub>,
- (f) -A<sub>28</sub>-A<sub>29</sub>-A<sub>30</sub>-A<sub>31</sub>-A<sub>32</sub>-R<sub>3</sub>,
- (g) -A<sub>28</sub>-A<sub>29</sub>-A<sub>30</sub>-A<sub>31</sub>-A<sub>32</sub>-A<sub>33</sub>-R<sub>3</sub>, and

(h)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-A_{33}-A_{34}-R_3;$

$R_{1a}$  is H, alkyl, aralkyl or  $-COR_2$ ;

5  $R_{1b}$  is  $R_{1a}$  or a group of formula



$R_2$  is alkyl, alkenyl, alkynyl, aryl or aralkyl;

10

$R_3$  is a group of formula  $A_{35}-OR_4$  or  $A_{35}-NR_4R_5$ ;

$R_4$  and  $R_5$  are independently H or lower alkyl;

15

$R_6$  and  $R_9$  are independently H or alkyl;

$R_7$  is alkyl;

$R_8$  is H, alkyl or  $COR_2$ ;

20

$R_{10}$  is H or halogen;

$R_{11}$  is alkyl or aralkyl;

25

$A_0$  is absent or a peptide of from one to six amino acid residues;

$A_1$  is Ser, Ala, Gly or D-Pro, or an equivalent amino acid thereof;

$A_2$  is Ala, Val or Gly, or an equivalent amino acid thereof;

30

A<sub>3</sub> is Ala, Ser, Gly or D-Pro, or an equivalent amino acid thereof;

A<sub>4</sub> is Glu, Ala or Gly, or an equivalent amino acid thereof;

5 A<sub>5</sub> is Ile, His, Ala or Gly, or an equivalent amino acid thereof;

A<sub>6</sub> is Ala, Gln, Gly or D-Pro, or an equivalent amino acid thereof;

A<sub>7</sub> is Ala, Leu or Gly, or an equivalent amino acid thereof;

10 A<sub>8</sub> is Leu, Nle, Gly or D-Pro, or an equivalent amino acid thereof;

A<sub>9</sub> is His, Ala, Gly or D-Pro, or an equivalent amino acid thereof;

15 A<sub>10</sub> is Ala, Asn, Gly Lys, Asp or D-Pro, or an equivalent amino acid thereof;

A<sub>11</sub> is Ala, Gly, Leu or Lys, or an equivalent amino acid thereof;

A<sub>12</sub> is Ala or Gly, or an equivalent amino acid thereof;

20 A<sub>13</sub> is Ala, Gly or Lys, or an equivalent amino acid thereof;

A<sub>14</sub> is Ala, Gly, His, Ser, Asp, Lys or D-Pro, or an equivalent amino acid thereof;

25 A<sub>15</sub> is Ala, Gly, Ile, D-Pro or Leu, or an equivalent amino acid thereof;

~~A<sub>16</sub> is Asn, Ala, Gly, D-Pro or Gln, or an equivalent amino acid thereof;~~

A<sub>17</sub> is Ala, Asp, Gly, Ser, Lys or D-Pro, or an equivalent amino acid thereof;

30 A<sub>18</sub> is Lys, or an equivalent amino acid thereof;

A<sub>19</sub> is Arg or Glu, or an equivalent amino acid thereof;

- A<sub>20</sub> is Arg, or an equivalent amino acid thereof;
- A<sub>21</sub> is Arg, Lys, Asp or Val, or an equivalent amino acid thereof;
- 5 A<sub>22</sub> is Asp, Lys, Orn or Glu, or an equivalent amino acid thereof;
- A<sub>23</sub> is Leu, Phe or Trp, or an equivalent amino acid thereof;
- A<sub>24</sub> is Leu, or an equivalent amino acid thereof;
- 10 A<sub>25</sub> is Arg, His, Asp, Lys or Glu, or an equivalent amino acid thereof;
- A<sub>26</sub> is Lys or His, or an equivalent amino acid thereof;
- 15 A<sub>27</sub> is Leu or Lys, or an equivalent amino acid thereof;
- A<sub>28</sub> is Ile or Leu, or an equivalent amino acid thereof;
- A<sub>29</sub> is Ala, Asp, Glu or Gln, or an equivalent amino acid thereof;
- 20 A<sub>30</sub> is Asp, Lys or Glu, or an equivalent amino acid thereof;
- A<sub>31</sub> is Ile, Leu or Val, or an equivalent amino acid thereof;
- 25 A<sub>32</sub> is His, or an equivalent amino acid thereof;
- A<sub>33</sub> is Asn or Thr, or an equivalent amino acid thereof; and
- 
- A<sub>34</sub> is Ala or Phe, or an equivalent amino acid thereof; and
- 30 A<sub>35</sub> is absent or a peptide of from 1 to 4 amino acids.

The foregoing peptide compounds are disclosed in WO 98/51324 to possess useful properties, more particularly pharmaceutical properties. They are especially useful for treating disease states

capable of being modulated by compounds which bind to parathyroid hormone receptors either with or without concomitant stimulation of adenylyl cyclase activity. The present invention is directed to an improved method for synthesizing these peptide compounds.

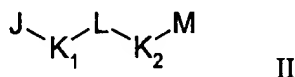
5           The solid phase synthesis of cyclic peptides typically involves the sequential addition of amino acids to a peptide synthesis resin to obtain a resin-bound peptide possessing all or a portion of the amino acid sequence of the desired cyclic peptide. The amino acid side chain residues to be cyclized are then deprotected and cyclization is effected. If cyclization is effected prior to completion of the entire amino acid sequence, the remaining amino acids are added and the completed peptide is then cleaved from the  
10 resin and purified.

          However, the linear approach to the preparation of peptides, and to cyclic peptides in general, is frequently inefficient and may therefore not be cost effective for the preparation of large quantities of peptide. In cases where a peptide is assembled in a linear fashion, the ease of purification of the peptide decreases as the number of amino acid residues in the peptide increases. Furthermore, for cyclic  
15 peptides, preparing side-chain bridge(s) near the end of the synthesis imparts the problems associated with preparing the side-chain bridge, including low yield, side-reactions and removal of impurities, to the entire peptide. Consequently, the incorporation of a cyclic unit in a lengthy peptide increases the difficulties inherent in linear peptide syntheses. A commercially feasible synthesis of cyclic hPTH analogues such as those disclosed in WO 98/51324 and set forth above requires a synthetic approach  
20 which overcomes both the complicating factors of length and cyclic components and which would enable preparation of commercially useful quantities in the context of drug manufacturing. The present invention is directed to a more efficient preparation of cyclic hPTH and hPTHrP analogues via a process using bridged and non-bridged fragments.

## 25 SUMMARY OF THE INVENTION

This invention is directed to a method of preparing cyclic hPTH and hPTHrP analogs of formula

30



wherein

J, L, and M are linear peptide fragments

K<sub>1</sub> is absent or a cyclic peptide fragment, and

$K_2$  is a cyclic peptide fragment;

this method comprising the steps of:

- 5 (1) preparing  $M-(R)$  by sequential attachment of suitably protected amino acid residues to a resin to provide:



- 10 where  $(R)$  is a suitable peptide synthesis resin and M is a polypeptide fragment

(2) preparing separately by conventional peptide synthesis N-terminal protected cyclic polypeptide fragment of formula IV



wherein P is a suitable amine protecting group,

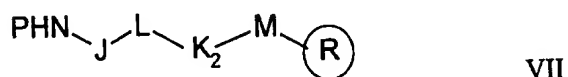
- 20 (3) Coupling III with IV to provide a peptide of formula V



- 25 (4) if  $K_2$  is absent, then (a) polypeptide fragments J and L are prepared as a single polypeptide of formula VI



and polypeptide VI is coupled to peptide V to provide a peptide of formula VII



5

or, optionally, (b) the protected individual amino acid elements of polypeptides J and L are added sequentially to the peptide fragment of formula V,

or, optionally, (c) either or both J and L are prepared separately as polypeptide fragments and coupled to the growing peptide starting with the fragment of formula V,

10

(5) when cyclic peptide  $\text{K}_1$  is present, then (a) a polypeptide fragment of formula VIII



15

wherein

P is a suitable amine protecting group,

20 is prepared separately by conventional peptide synthesis procedures,

(b) a peptide fragment of formula IX

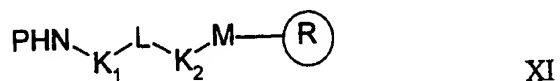


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is prepared and coupled with peptide fragment V to provide a peptide fragment of formula X



and (c) the cyclic peptide fragment VIII is coupled with the peptide fragment of formula X to provide a peptide fragment of formula XI



(d) peptide fragment of formula XII is prepared



and fragment XII is coupled with fragment XI, and  
(6) cleaving the resin and deprotecting.

In the process described herein, cyclic peptide fragments are prepared separately for coupling with a resin or resin-bound peptide. The separate preparation of the cyclic peptide fragment allows for the convergent synthesis of resin bound cyclic peptides, resulting in increased yield and throughput of the resulting cyclic peptide. Difficulties associated with preparing the bridged fragment are confined to a smaller peptide subunit, which can be purified prior to coupling with the solid phase synthesis resin. The practice of this technique in the context of certain preferred hPTH and hPTHrP analogs requires the synthesis of new peptide fragments and new sequences of events.

## DETAILED DESCRIPTION OF THE INVENTION

As used above and throughout the specification, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

### Definitions of Terms

"Alkyl" means an aliphatic hydrocarbon group which may be straight or branched having about 1 to about 20 carbon atoms in the chain. Branched means that one or more lower alkyl groups are attached to a linear alkyl chain. "Lower alkyl" means about 1 to 4 carbon atoms in the chain which may be straight or branched. Alkyl groups are exemplified by methyl, ethyl, *n*- and *iso*-propyl, *n*-, *sec*-, *iso*- and *tert*-butyl, and the like.

"Alkenyl" means aliphatic hydrocarbon group containing a carbon-carbon double bond and which may be straight or branched having about 2 to about 20 carbon atoms in the chain. "Lower alkenyl" means about 2 to 4 carbon atoms in the chain which may be straight or branched. Exemplary

alkenyl groups include ethenyl, propenyl, *n*-butenyl, *i*-butenyl, 3-methylbut-2-enyl, *n*-pentenyl, heptenyl, octenyl, cyclohexylbutenyl and decenyl.

“Alkynyl” means aliphatic hydrocarbon group containing a carbon-carbon triple bond and which may be straight or branched having about 2 to about 20 carbon atoms in the chain. “Lower alkynyl” means about 2 to 4 carbon atoms in the chain which may be straight or branched. Exemplary alkynyl groups include ethynyl, propynyl, *n*-butynyl, 3-methylbut-2-ynyl, *n*-pentynyl, heptynyl, octynyl and decynyl.

“Alkylene” means a divalent group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, for example methylene, 1,2-ethylene, 1,1-ethylene, 1,3-propylene, 2,2-dimethylpropylene, and the like.

“Phenylalkyl” means a phenyl group attached to the parent molecular moiety through an alkylene group. The alkylene group is preferably of about 1 to about 7 carbon atoms. Representative phenylalkyl groups include benzyl, 2-phenylethyl, 2-propylphenyl, and the like.

15

“Amine protecting group” means an easily removable group which is known in the art to protect an amino group against undesirable reaction during synthetic procedures and to be selectively removable. The use of N-protecting groups is well known in the art for protecting groups against undesirable reactions during a synthetic procedure and many such protecting groups are known, cf, for example, T.H. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2nd edition, John Wiley & Sons, New York (1991), incorporated herein by reference. Preferred N-protecting groups are acyl, including formyl, acetyl, chloroacetyl, trichloroacetyl, *o*-nitrophenylacetyl, *o*-nitrophenoxyacetyl, trifluoroacetyl, acetoacetyl, 4-chlorobutyryl, isobutyryl, *o*-nitrocinnamoyl, picolinoyl, acylisothiocyanate, aminocaproyl, benzoyl and the like. and acyloxy including methoxycarbonyl, 9-fluorenylmethoxycarbonyl, 2,2,2-trifluoroethoxycarbonyl, 2-trimethylsilyl ethoxycarbonyl, vinylloxycarbonyl, allyloxycarbonyl, *t*-butyloxycarbonyl (BOC), 1,1-dimethylpropynyloxycarbonyl, benzyloxycarbonyl (CBZ), *p*-nitrophenylsulfinyl, *p*-nitrobenzyloxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, allyloxycarbonyl (Alloc), and the like.

“Amino acid” means an amino acid selected from the group consisting of natural and unnatural amino acids as defined herein. The amino acids may be neutral, positive or negative depending on the substituents in the side chain. “Neutral amino acid” means an amino acid containing uncharged side chain substituents. Exemplary neutral amino acids include alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine and cysteine. “Positive amino acid” means an amino acid in which the side chain substituents are positively charged at physiological pH.

Exemplary positive amino acids include lysine, arginine and histidine. "Negative amino acid" means an amino acid in which the side chain substituents bear a net negative charge at physiological pH.

Exemplary negative amino acids include aspartic acid and glutamic acid. Preferred amino acids are  $\alpha$ -amino acids. The most preferred amino acids are  $\alpha$ -amino acids having L stereochemistry at the  $\alpha$ -carbon.

"Amino acid residue" means the individual amino acid units incorporated into a peptide or peptide fragment.

"Natural amino acid" means an  $\alpha$ -amino acid selected from the group consisting of alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, lysine, arginine, histidine, aspartic acid and glutamic acid.

"Unnatural amino acid" means an amino acid for which there is no nucleic acid codon.

Examples of unnatural amino acids include, for example, the D-isomers of the natural  $\alpha$ -amino acids as indicated above; Aib (aminobutyric acid), bAib (3-aminoisobutyric acid), Nva (norvaline),  $\beta$ -Ala, Aad (2-aminoadipic acid), bAad (3-aminoadipic acid), Abu (2-aminobutyric acid), Gaba ( $\gamma$ -aminobutyric acid), Acp (6-aminocaproic acid), Dbu (2,4-diaminobutyric acid),  $\alpha$ -aminopimelic acid, TMSA (trimethylsilyl-Ala), alle (allo-isoleucine), Nle (norleucine), *tert*-Leu, Cit (citrulline), Orn, Dpm (2,2'-diaminopimelic acid), Dpr (2,3-diaminopropionic acid),  $\alpha$ - or  $\beta$ -Nal, Cha (cyclohexyl-Ala), hydroxyproline, Sar (sarcosine), and the like; cyclic amino acids;  $N^{\alpha}$ -alkylated amino acids such as MeGly ( $N^{\alpha}$ -methylglycine), EtGly ( $N^{\alpha}$ -ethylglycine) and EtAsn ( $N^{\alpha}$ -ethylasparagine); and amino acids in which the  $\alpha$ -carbon bears two side-chain substituents.

"Equivalent amino acid" means an amino acid which may be substituted for another amino acid in the peptides according to the invention without any appreciable loss of function. In making such changes, substitutions of like amino acids is made on the basis of relative similarity of side chain substituents, for example regarding size, charge, hydrophilicity, hydropathicity and hydrophobicity as described herein.

As detailed in United States Patent No. 4,554,101, incorporated herein by reference, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4). It is understood that an amino acid residue can be substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0) and still obtain a biologically equivalent polypeptide.

In a similar manner, substitutions can be made on the basis of similarity in hydropathic index. Each amino acid residue has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those hydropathic index values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe

(+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5). In making a substitution based on the hydropathic index, a value of within plus or minus 2.0 is preferred.

5 "Peptide" and "polypeptide" mean a polymer in which the monomers are amino acid residues joined together through amide bonds. Preferred peptides of the present invention are those comprising  $\alpha$ -amino acids. The most preferred peptides of the present invention comprise  $\alpha$ -amino acids having L stereochemistry at the  $\alpha$ -carbon.

"Cyclic peptide" means a peptide containing one or more cyclic peptide fragments as defined herein.

10 "Peptide fragment" means a peptide subunit of the parent peptide. "Peptide fragment" may imply a linear, branched, or cyclic subunit of the target peptide.

"Linear Peptide" means a peptide or polypeptide in which the amino acids are linked to one another via an amid bond between the N-terminal of one and the C-terminal or another.

15 "Branched peptide" means a peptide or polypeptide within which one or several constituent individual amino acids bearing carboxylic acid or amine side chains are attached to another peptide substituent via these side chains.

"Cyclic peptide fragment" means a peptide fragment as defined herein in which a substituent on one amino acid residue is linked to a substituent on another amino acid residue in the peptide fragment. The linking is preferably between the side chains of two amino acid residues in the peptide fragment, 20 preferably through an ester, amide, disulfide or lanthionine bond. The bonding between the two amino

acid side chains is designated herein as



The ester, amide, disulfide or lanthionine bond which links two amino acid residues of the cyclic peptide is formed between the side-chain functionalities. Thus, an amide is bond is formed between the side-chain carboxyl group of an acidic amino acid residue and the side-chain amino group of a basic amino acid residue; ester bonds are formed between the side-chain carboxyl group of an acidic amino acid residue and the side-chain hydroxyl group of a hydroxyl-containing amino acid residue; disulfides 25 are formed from amino acid residues containing side chain sulfhydryl groups; and lanthionine bridges are formed by desulfurization of the corresponding disulfide.

30 The number of atoms in the bridge resulting from the amide, ester, disulfide or lanthionine bond formed as described above will vary depending on the length of the side chain and the type of bond (ie, amide, ester, disulfide or lanthionine). The bridge preferably comprises from 2 to 12 atoms, more preferably from 6 to 10 atoms. The most preferred number of atoms contained in the bridge is 7, this

bridge preferably comprising an amide bond between the side-chain functionalities of a Lys and an Asp residue.

"Resin" means a solid support modified with a reactive group such that the solid support is amenable to coupling with the carboxy or N-terminus of an amino acid, peptide, or cyclic peptide fragment as defined herein. Representative resins include Merrifield resin (chloromethylated polystyrene), hydroxymethyl resin, 2-chlorotrityl chloride resin, trityl chloride resin, Rink acid resin (4-benzyloxy-2',4'-dimethoxybenzhydrol resin), trityl alcohol resin, PAM resin (4-hydroxymethyl-phenylacetamidomethyl resin), Wang resin (p-benzyloxybenzyl alcohol resin), MBHA resin (p-methylbenzhydrylamine resin), BHA resin (benzhydrylamine resin), Rink amide resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy resin) and PAL resin (5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid-MBHA resin). Preferred resins are chlorotrityl resin, Rink acid resin and Rink amide resin.

"Solid support" means a substrate which is inert to the reagents and reaction conditions described herein, as well as being substantially insoluble in the media used. Representative solid supports include inorganic substrates such as kieselguhr, silica gel, and controlled pore glass; organic polymers including polystyrene, including 1-2% copolystyrene divinyl benzene (gel form) and 20-40% copolystyrene divinyl benzene (macro porous form), polypropylene, polyethylene glycol, polyacrylamide, cellulose, and the like; and composite inorganic/polymeric compositions such as polyacrylamide supported within a matrix of kieselguhr particles. See J.M. Stewart and J.D. Young, *Solid Phase Peptide Synthesis*, 2nd. Ed., Pierce Chemical Co. (Chicago, IL, 1984).

In addition, "solid support" includes a solid support as described above which is affixed to a second inert support such as the pins described in Technical Manual, Multipin™ SPOC, Chiron Technologies (1995) and references therein which comprise a detachable polyethylene- or polypropylene-based head grafted with an amino functionalized methacrylate copolymer and an inert stem.

In addition, "solid support" includes polymeric supports such as the polyethylene glycol supports described by Janda et al., *Proc. Natl. Acad. Sci. USA*, 92, 6419-6423 (1995) and S. Brenner, WO 95/16918, which are soluble in many solvents but can be precipitated by the addition of a precipitating solvent.

The names of natural and unnatural amino acids and residues thereof used herein follow the naming conventions suggested by the IUPAC Commission on the Nomenclature of Organic Chemistry and the IUPAC-IUB Commission on Biochemical Nomenclature as set out in "Nomenclature of  $\alpha$ -Amino Acids (Recommendations, 1974)" *Biochemistry*, 14(2), (1975). To the extent that the names and

abbreviations of amino acids and residues thereof employed in this specification and appended claims differ from those noted, differing names and abbreviations will be made clear.

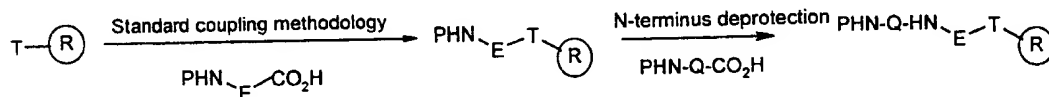
A representative peptide prepared according to the process of this invention is denoted, for example, as cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>, Nle<sup>8</sup>, Lys<sup>18</sup>, Asp<sup>22</sup>, Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub> with the linked amino acid residues in the parenthesis following "cyclo" and with substituted amino acids from the natural sequence placed in brackets. hPTH stands for human PTH and PTHrP for human parathyroidhormone-related protein. The numbers in the second parenthesis refer to the number of amino acid residues in the peptide, beginning at the N-terminus (ie, the first 31 amino acids of hPTH).

## 10 Preferred Embodiments

As shown in Scheme 1, the preparation of a resin-bound cyclic peptide involves coupling cyclic and noncyclic peptide fragments to a resin, resin bound amino acid, or resin bound peptide.

Scheme 1

15



(R) is a resin suitable for peptide synthesis.

T is an H<sub>2</sub>N, and amino acid, or a cyclic or acyclic peptide.

20 E and Q are each or independently an N terminal protected amino acid, or an N-terminal protected cyclic or acyclic peptide fragment.

The coupling is preferably accomplished between the carboxy terminus of the peptide fragment and the resin, resin-bound amino acid or resin bound peptide. When the peptide fragment is coupled to a resin-bound amino acid or peptide, the coupling is preferably through an amide bond between the carboxy terminus of the cyclic peptide fragment and the N-terminus of the resin-bound amino acid or peptide.

In order for the coupling reaction to proceed, the carboxyl group of the peptide fragment must be activated. Many methods of activation may be used in the practice of the invention and include, for example, preformed symmetrical anhydrides (PSA), preformed mixed anhydride (PMA), acid chlorides, active esters, and in situ activation of the carboxylic acid, as set forth in Fields and Noble, 1990, "Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids", Int. J. Pept. Protein Res. 35:161-214.

Representative activating agents include isopropyl chloroformate, diisopropylcarbodiimide (DIC), DIC admixed with 1-hydroxybenzotriazole (HOBT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl), benzotriazole-1-yloxy-tris((dimethylamino)phosphonium)hexafluorophosphate (BOP), 5 benzotriazole-1-yloxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBROP), O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU), 2-(1H-benzotriazole-1-yl)-1.1.3.3-tetramethyluronium tetrafluoroborate (TBTU), 2-(1H-benzotriazole-1-yl)-1.1.3.3-tetramethyluronium hexafluorophosphate (HBTU), 10 2-[2-oxo-1-(2H)-pyridyl]-1,1,3,3-bis(pentamethylenuronium) tetrafluoroborate (TOPPipU), N,N'-dicyclohexylcarbodiimide (DCC), DCC admixed with HOBT, and the like. Suitable solvents for the coupling reaction include dichloromethane, DMF, DMSO, toluene, THF, and the like. Coupling times range from about 1 to about 48 hours, depending upon the resin and carboxylic acid derivative to be coupled, activating agent, solvent and temperature. The coupling is accomplished at from about -10 15 °C to about 50 °C, preferably at about ambient temperature.

In order to prevent interference with the above-described coupling reaction, the N-terminus of the cyclic peptide fraction is protected by an acid or base sensitive group. Such protecting groups should have the properties of being stable to the conditions of amide bond formation, while being readily removable without destruction of the growing peptide chain or racemization of any of the chiral centers 20 contained therein. Suitable protecting groups are 9-fluorenylmethyloxycarbonyl (Fmoc), *t*-butyloxycarbonyl (Boc), benzyloxycarbonyl (Cbz), biphenylisopropylloxycarbonyl, *t*-amyloxycarbonyl, isobornyloxycarbonyl, ( $\alpha,\alpha$ )dimethyl-3,5-dimethoxybenzyloxycarbonyl, *o*-nitrophenylsulfonyl, 2-cyano-*t*-butyloxycarbonyl, and the like. The 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group is preferred.

25 Likewise, it may be necessary to protect any reactive side-chain functional groups in the cyclic peptide fragment. Particularly preferred side chain protecting groups are, for side chain amino groups as in lysine and arginine: 2,2,5,7,8-pentamethylchroman-6-sulfonyl (pmc), nitro, *p*-toluenesulfonyl, 4-methoxybenzenesulfonyl, Cbz, Boc, Alloc (allyloxycarbonyl) and adamantyloxycarbonyl; for tyrosine: benzyl, *o*-bromobenzyloxycarbonyl, 2,6-dichlorobenzyl, isopropyl, *t*-butyl (*t*-Bu), cyclohexyl, 30 cyclophenyl and acetyl (Ac); for serine: *t*-butyl, benzyl and tetrahydropyranyl; for histidine: trityl, benzyl, Cbz, *p*-toluenesulfonyl and 2,4-dinitrophenyl; for tryptophan: formyl and Boc; for asparagine and glutamine: Trt (trityl); for aspartic acid and glutamic acid: O-*t*-Bu and OAllyl and Obenzyl.

Coupling of the fragments to the resin or resin bound peptide is preferably accomplished at about ambient temperature using about 2 molar equivalents (relative to resin) of the Fmoc-protected cyclic

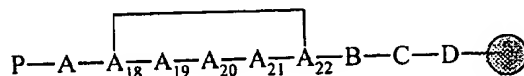
peptide fragment, activated with about an equimolar portion of benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP) and hydroxybenzotriazole hydrate in the presence of about 4 equivalents of diisopropylethyl amine in dimethylformamide over about 16-48 hours.

The completeness of coupling should be assessed. Those skilled in the art would be familiar with the well known monitoring tests such as ninhydrin (the Kaiser test), picric acid, 2,4,6-trinitrobenzenesulfonic (TNBS), fluorescamine, and chloranil, which are based on reagent reaction with free amino groups to produce a chromophoric compound. If imino acids (e.g., Pro and Hyp) are used, isatin monitoring is a preferred method. Fields and Noble, *supra*. Quantification of reaction completeness may be monitored during the course of the reaction, e.g., as described by Salisbury et al. (International Patent Publication No. WO91/03485).

With Fmoc synthesis, the Kaiser and TNBS tests are preferred. In the Kaiser test, a sample of resin peptide can be tested with ninhydrin reagent obtained from Pierce Chemical in the method set forth by Sarin et al. (1981, Anal. Biochem. 117:147-157.). Similarly, a sample of resin peptide can be tested using trinitrobenzenesulfonic acid (TNBS), available from Fluka, as set forth by Takajashi (1984, Chem Lett. 1: 127 )


If the coupling reaction is incomplete as determined by one or the other of these tests, the reaction can be forced to completion by several methods familiar to those in the art, including (a) a repeat coupling using a one to five fold excess of protected amino acid, (b) a repeat coupling using different or additional solvents (e.g., trifluoroethane), or (c) the addition of chaotropic salts, e.g., NaClO<sub>4</sub> or LiBr (Klis and Stewart, 1990, "Peptides: Chemistry, Structure and Biology," Rivier and Marshall, eds., ESCOM Publ., p. 904-906).

A Preferred resin-bound cyclic peptide fragment has formula II



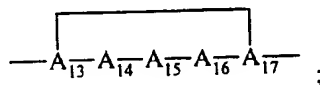
II

wherein

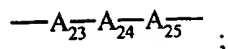
 is a resin;

P is an amine protecting group;

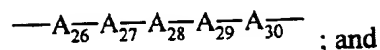
A is absent or a group of formula



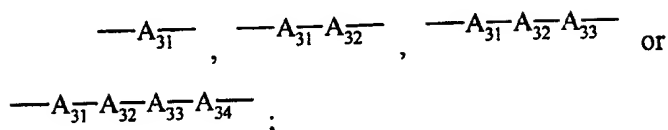
B is a group of formula



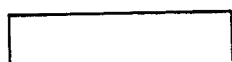
C is a group of formula



D is



5 wherein



is a lactam, ester, disulfide or lanthionine bridge;  $A_{13}$ ,  $A_{17}$ ,  $A_{18}$  and  $A_{22}$  are amino acid residues;  $A_{14}$  is His(Trt) or Ser(tBu);  $A_{15}$  and  $A_{28}$  are independently Ile or Leu; Ser(tBu) or Leu;  $A_{16}$  is Asn(Trt) or Gln(Trt);  $A_{19}$  is Arg(Pmc) or Glu(OtBu);  $A_{20}$  is Arg(Pmc);  $A_{21}$  is Arg(Pmc) or Val;  $A_{23}$  is Phe or Trp(Boc);  $A_{24}$  is Leu;  $A_{25}$  is Arg(Pmc) or His(Trt);  $A_{26}$  and  $A_{30}$  are amino acid residues wherein the side chains of  $A_{26}$  and  $A_{30}$  are optionally linked through an amide, disulfide or lanthionine bond;  $A_{27}$  is Leu or Lys(Boc);  $A_{29}$  is Ala or Gln(Trt);  $A_{31}$  is Ile or Val;  $A_{32}$  is His(Trt);  $A_{33}$  is Asn(Trt) or Thr(tBu); and  $A_{34}$  is Ala or Phe.

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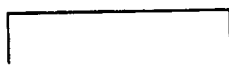
Another preferred resin-bound cyclic peptide fragment has formula II wherein



15

is a lactam bridge and the side chains of  $A_{26}$  and  $A_{30}$  are optionally linked through an amide bond.

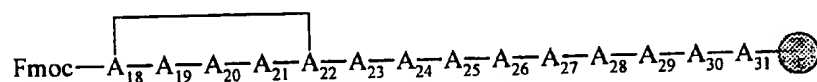
Another preferred resin-bound cyclic peptide has formula II wherein



is a lactam bridge;  $A_{13}$  and  $A_{18}$  are Lys;  $A_{17}$  and  $A_{22}$  are Asp; and  $A_{26}$  is Lys(Boc); and  $A_{30}$  is Asp(OtBu); or the side chains of  $A_{26}$  and  $A_{30}$  are optionally linked through an amide bond.

20


Another preferred resin-bound cyclic peptide fragment has formula III



25

III

wherein

 is a lactam bridge;

A<sub>18</sub> is Lys; A<sub>19</sub> is Glu(OtBu); A<sub>20</sub> is Arg(Pmc); A<sub>21</sub> is Val; A<sub>22</sub> is Asp; A<sub>23</sub> is Trp(Boc); A<sub>24</sub> is Leu; A<sub>25</sub> is Arg(Pmc); A<sub>26</sub> is Lys(Boc); A<sub>27</sub> is Leu; A<sub>28</sub> is Leu; A<sub>29</sub> is Gln(Trt); A<sub>30</sub> is Asp(OtBu); and A<sub>31</sub> is Val.

5

The resin-bound peptide prepared as described above may be further elaborated, for example by coupling with one or more additional cyclic peptide fragments, by coupling with one or more peptide fragments, by sequential addition of individual amino acids, or by any combination of the foregoing.

10

The complete cyclic peptide is then cleaved from the resin and purified. Protecting groups may be removed prior or subsequent to, or simultaneously with, cleavage from the resin. The fully deprotected peptide may purified, alone or in combination, by acid-base extraction, recrystallization, lyophilization, by a sequence of chromatographic steps employing any or all of the following types: ion exchange on a weakly basic resin in the acetate form; hydrophobic adsorption chromatography on underivitized polystyrene-divinylbenzene (for example,

15

AMBERLITE® XAD); silica gel adsorption chromatography; ion exchange chromatography on carboxymethylcellulose; partition chromatography, e.g. on SEPHADEX® G-25, LH-20 or countercurrent distribution; high performance liquid chromatography (HPLC), especially reverse-phase HPLC on octyl- or octadecylsilyl-silica bonded phase column packing.

20

#### Preparation of the Peptide Fragment

The fragment is prepared by synthesizing the peptide backbone, which entails assembling the amino acids comprising the fragment in their proper order. The peptide backbone of the fragment may be synthesized by any of the techniques that are known to those skilled in the art. For solid phase peptide synthesis, a summary of the many techniques may be found in J.M. Stewart and J.D. Young, *Solid Phase Peptide Synthesis*, W.H. Freeman Co. (San Francisco), 1963 and J. Meienhofer, *Hormonal Proteins and Peptides*, vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, *The Peptides*, vol. 1, Academic Press (New York), 1965.

25

30

As would be known to those of ordinary skill in the art, the process of peptide synthesis on solid supports generally involves building a peptide from the carboxyl or C-terminal end in which the C-terminal amino acid with its  $\alpha$ -amino group protected is attached to a solid phase polymer. The

N-protecting group is then cleaved off, and the next amino acid, also N-protected, is coupled by a peptide bond to the  $\alpha$ -amino group of the amino acid attached to the solid support as described above. The cycle of deprotection of the prior amino acid and coupling the additional amino acid is repeated until the peptide is completed. Any reactive side chains of the amino acids are protected by chemical groups that can withstand the coupling and N<sup>α</sup>-deprotection procedure but can be removed at the end of the synthesis. When the peptide fragment is prepared using solid phase methods, the fragment is obtained for use in couplings to other fragments by cleavage from the resin.

A cyclic fragment is prepared in similar fashion. However, backbone assembly is followed by (1)selective deprotection of the side-chain functionalities to be cyclized, (2)cyclization, and (3)optional removal of any remaining protecting groups.

The use of Fmoc amino acids is but one strategy of peptide synthesis. A Boc (t-butyloxycarbonyl-protected amino group) strategy may also be used to prepare a peptide bound to the solid support (e.g., Geysen et al., 1987, J. Immunol. Methods 102:259-274.)

Amino acids used for peptide synthesis may be standard Boc (N<sup>α</sup>-amino protected N<sup>α</sup>-t-butyloxycarbonyl) amino acids described by Merrifield (1963, J. Am. Chem. Soc. 85:2149-2154), or the base-labile N<sup>α</sup>-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han (1972, J. Org. Chem. 37:3403-3409). Both Fmoc and Boc N<sup>α</sup>-amino protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art. In addition, the method of the invention can be used with other N<sup>α</sup>-protecting groups that are familiar to those skilled in this art. Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, IL; Fields and Noble, 1990, Int. J. Pept. Protein Res. 35:161-214, or using automated synthesizers, such as sold by ABS.

Although C-terminal to N-terminal peptide synthesis is the standard, those of ordinary skill in the art will recognize that the peptide fragment is also amenable to synthesis from N-terminus to C-terminus.

#### Preparation of the Lactam-Bridge

Lactam-bridged cyclic peptide fragments are prepared by formation of an amide bond between the side-chain carboxyl group of an acidic amino acid residue and the side-chain amino group of a basic amino acid residue in the presence of an activating agent as described above. Preferred acidic amino

acid residues include Asp, Glu,  $-\text{NHCH}[(\text{CH}_2)_3\text{CO}_2\text{H}]\text{CO}-$  and  $-\text{NHCH}[(\text{CH}_2)_4\text{CO}_2\text{H}]\text{CO}-$ , Asp being most preferred. Preferred basic amino acid residues include His, Lys, Orn,  $-\text{NHCH}(\text{CH}_2\text{NH}_2)\text{CO}-$  and  $-\text{NHCH}[(\text{CH}_2)_2\text{NH}_2]\text{CO}-$ , Lys being most preferred.

In instances wherein the peptide precursor to the cyclic peptide fragment contains more than one  
 5 acidic or basic amino acid residue, protecting groups for the additional acidic or basic amino acids in the cyclic peptide fragment are selected so that the amino acids to be cyclized may be selectively deprotected. Preferably, the desired acidic and basic amino acid residues are deprotected simultaneously. Furthermore, in addition to being stable to the reagents used to deprotect the selected basic and acidic amino acid residues, the protecting groups on the remaining amino acid residues are selected to be stable  
 10 to the cyclization conditions employed.

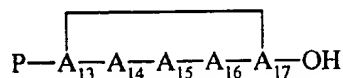
The term "orthogonality" when used in reference to side chain protecting groups refers to a situation as described herein in which there are two or more classes of protecting groups on a molecule, each class most optimally removed under specific conditions, while remaining stable to conditions used to remove protecting groups in other classes. Thus one can remove all protecting groups of one class,  
 15 while leaving all others intact.

Preferred protecting groups having the desired orthogonality are: for the acidic amino acid residue to be cyclized: allyl; for the basic amino acid residue to be cyclized: allyloxycarbonyl (alloc); for any additional acidic amino acid residues: *tert*-butyl (tBu); and for any additional basic amino acid residues: *tert*-butoxycarbonyl (Boc).

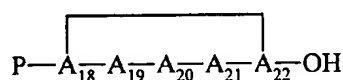
20 Following synthesis of the peptide backbone of the cyclic peptide fragment using solid phase or solution phase techniques, the allyl and allyloxycarbonyl protecting groups are removed simultaneously by treatment with palladium, preferably tetrakis(triphenylphosphine) palladium(0) in the presence of *N*-methylaniline. Formation of the lactam bridge is then accomplished as described herein for amide bond formation.

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Preferred cyclic peptide fragments for use in preparing the resin-bound cyclic peptides of this invention have formula XV, XVI or XVII

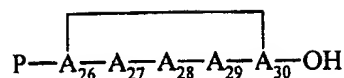


XV



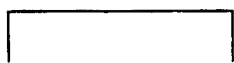
XVI

30



XVII

wherein

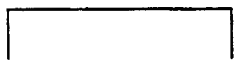


is a lactam, disulfide or lanthionine bridge;

5 P is an amine protecting group;

$\text{A}_{13}$ ,  $\text{A}_{17}$ ,  $\text{A}_{18}$ ,  $\text{A}_{22}$ ,  $\text{A}_{26}$  and  $\text{A}_{30}$  are amino acid residues;  $\text{A}_{14}$  is His(Trt) or Ser(tBu);  $\text{A}_{15}$  is Ser(tBu) or Leu;  $\text{A}_{16}$  is Asn(Trt) or Gln(Trt);  $\text{A}_{19}$  is Arg(Pmc) or Glu(OtBu);  $\text{A}_{20}$  is Arg(Pmc);  $\text{A}_{21}$  is Arg(Pmc) or Val;  $\text{A}_{27}$  is Leu or Lys(Boc);  $\text{A}_{28}$  is Ile or Leu; and  $\text{A}_{29}$  is Ala or Gln(Trt).

10 More preferred cyclic peptide fragments have formula IV, V or VI wherein



is a lactam bridge;

P is Fmoc;

$\text{A}_{13}$ ,  $\text{A}_{18}$  and  $\text{A}_{26}$  are Lys;  $\text{A}_{17}$ ,  $\text{A}_{22}$  and  $\text{A}_{30}$  are Asp;  $\text{A}_{14}$  is His(Trt) or Ser(tBu);  $\text{A}_{15}$  is Ser(tBu) or Leu;  $\text{A}_{16}$  is Asn(Trt) or Gln(Trt);  $\text{A}_{19}$  is Arg(Pmc) or Glu(OtBu);  $\text{A}_{20}$  is Arg(Pmc);  $\text{A}_{21}$  is Arg(Pmc) or Val;  $\text{A}_{27}$

15 is Leu or Lys(Boc);  $\text{A}_{28}$  is Ile or Leu; and  $\text{A}_{29}$  is Ala or Gln(Trt).

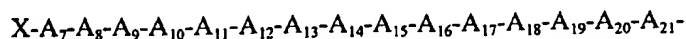
A still more preferred cyclic peptide fragment for use in preparing the resin-bound cyclic peptides of this invention is Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-OH.

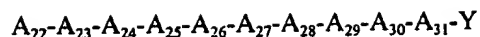
20 Cyclic peptide parathyroid hormone analogs and parathyroid hormone-related protein analogs suitable for preparation using the process of this invention are disclosed by:

1. Condon et al., U.S. Ser. No. 60/046,472 (filed May 14, 1997);
2. Willick et al., U.S. Pat. No.: 5,556,940 (Sept. 17, 1997);
3. Chorev et al., U.S. Pat. No. 5,717,062 (Feb. 10, 1998);
- 25 4. Vickery et al., WO 96/40775 (published Dec. 19, 1996).

A preferred cyclic peptide suitable for preparation using the process of this invention has formula I

30





I

wherein

- 5 X is selected from the group consisting of (a)  $R_{18}-A_1-A_2-A_3-A_4-A_5-A_6-$   
 (b)  $R_{16}-A_2-A_3-A_4-A_5-A_6-$ , (c)  $R_{16}-A_3-A_4-A_5-A_6-$ , (d)  $R_{16}-A_4-A_5-A_6-$ , (e)  $R_{16}-A_5-A_6-$ ,  
 (f)  $R_{16}-A_6-$  and (g)  $R_{16}-$  wherein  $R_{18}$  is selected from the group consisting of (1) H, (2) a peptide  
 comprising from 1 to 6 amino acids, (3) alkyl, (4) phenylalkyl, (5)  $-COR_2$  wherein  $R_2$  is selected from  
 alkyl, alkenyl, alkynyl, phenyl, naphthyl and phenylalkyl,  $R_{16}$  is selected from the group consisting of (1)  
 10 H, (2) alkyl, (3) phenylalkyl and (4)  $-COR_2$  wherein  $R_2$  is selected from alkyl, alkenyl, alkynyl, phenyl,  
 naphthyl and phenylalkyl,  $A_1$  is selected from Ser and Ala or an equivalent amino acid residue thereof,  
 $A_2$ ,  $A_3$ ,  $A_4$  and  $A_6$  are amino acid residues and  $A_5$  is selected from Ile and His or an equivalent amino acid  
 residue thereof;
- $A_7$ ,  $A_9$ ,  $A_{11}$  and  $A_{12}$  are amino acid residues;
- 15  $A_8$  is Leu or Nle or an equivalent amino acid residue thereof;  
 $A_{10}$  is Asp or Asn or an equivalent amino acid residue thereof;  
 $A_{13}$  and  $A_{17}$  are amino acid residues wherein the side chains of  $A_{13}$  and  $A_{17}$  are linked through an  
 amide bond;
- $A_{14}$  is His or Ser or an equivalent amino acid residue thereof;
- 20  $A_{15}$  and  $A_{28}$  are independently Ile or Leu or an equivalent amino acid residue thereof.  
 $A_{16}$  is Asn or Gln or an equivalent amino acid residue thereof;  
 $A_{18}$  and  $A_{22}$  are amino acid residues wherein the side chains of  $A_{18}$  and  $A_{22}$  are linked by an amide  
 bond;
- $A_{19}$  is Arg or Glu or an equivalent amino acid residue thereof;
- 25  $A_{20}$  is Arg or an equivalent amino acid residue thereof;  
 $A_{21}$  is Arg or Val or an equivalent amino acid residue thereof;  
 $A_{23}$  is Phe or Trp or an equivalent amino acid residue thereof;
- 
- $A_{24}$  is Leu or an equivalent amino acid residue thereof;
- $A_{25}$  is Arg or His or an equivalent amino acid residue thereof;
- 30  $A_{26}$  and  $A_{30}$  are amino acid residues wherein the side chains of  $A_{26}$  and  $A_{30}$  are optionally linked  
 through an amide bond;
- $A_{27}$  is Leu or Lys or an equivalent amino acid residue thereof;  
 $A_{29}$  is Ala or Gln or an equivalent amino acid residue thereof;  
 $A_{31}$  is Ile or Val or an equivalent amino acid residue thereof;

Y is selected from the group consisting of (a) R<sub>3</sub>, (b) A<sub>32</sub>-R<sub>3</sub>, (c) -A<sub>32</sub>-A<sub>33</sub>-R<sub>3</sub> and (d)-A<sub>32</sub>-A<sub>33</sub>-A<sub>34</sub>-R<sub>3</sub> wherein R<sub>3</sub> is -OH or -NR<sub>4</sub>R<sub>5</sub> wherein R<sub>4</sub> and R<sub>5</sub> are independently selected from hydrogen and lower alkyl, A<sub>32</sub> is His or an equivalent amino acid residue thereof, A<sub>33</sub> is Asn or Thr or an equivalent amino acid residue thereof and A<sub>34</sub> is Ala or Phe or an equivalent amino acid residue thereof.

5

Peptides of formula I possess useful pharmaceutical properties. They are especially useful for treating disease states capable of being modulated by compounds which bind to parathyroid hormone receptors either with or without concomitant stimulation of cAMPase activity. See U.S. Ser. No. 60/046,472.

10

Representative preferred cyclic peptides suitable for preparation using the process of this invention include, but are not limited to

- cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,2</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
15 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,3</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,4</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,5</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,6</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,7</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
20 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,10</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,11</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,12</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,13</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
25 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,14</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,15</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,16</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  


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bicyclo(Lys<sup>13</sup>-Asp<sup>17</sup>,Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>17,22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
bicyclo(Lys<sup>18</sup>-Asp<sup>22</sup>,Lys<sup>26</sup>-Asp<sup>30</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
30 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-34)NH<sub>2</sub>  
bicyclo(Lys<sup>13</sup>-Asp<sup>17</sup>,Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>17,22</sup>,Leu<sup>27</sup>]hPTH(1-34)NH<sub>2</sub>  
bicyclo(Lys<sup>18</sup>-Asp<sup>22</sup>,Lys<sup>26</sup>-Asp<sup>30</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-34)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Lys<sup>18</sup>,Asp<sup>22</sup>]hPTHrP(1-34)NH<sub>2</sub>  
cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Lys<sup>18,26,30</sup>,Asp<sup>22</sup>,Leu<sup>23,28,31</sup>,Glu<sup>25,29</sup>]hPTHrP(1-34)NH<sub>2</sub>

cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(7-34)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Lys<sup>18</sup>,Asp<sup>22</sup>]hPTHrP(7-34)NH<sub>2</sub>  
 bicyclo(Lys<sup>13</sup>,Asp<sup>17</sup>,Lys<sup>18</sup>-Asp<sup>22</sup>)[Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(7-34)NH<sub>2</sub>, and  
 bicyclo(Lys<sup>18</sup>-Asp<sup>22</sup>,Lys<sup>26</sup>-Asp<sup>30</sup>)[Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(7-34)NH<sub>2</sub>.

5

Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein the amino acid residues are  $\alpha$ -amino acid residues having L stereochemistry at the  $\alpha$ -carbon.

- 10 Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein X is selected from the group consisting of
- (a) R<sub>1a</sub>-A<sub>1</sub>-A<sub>2</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>- wherein R<sub>1a</sub> is H or Pro, A<sub>1</sub> is Ser or Ala, A<sub>2</sub> is Val, A<sub>3</sub> is Ser, A<sub>4</sub> is Glu, A<sub>5</sub> is Ile or His, A<sub>6</sub> is Gln, (b) R<sub>1b</sub>-A<sub>2</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>- wherein A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub> and A<sub>6</sub> are defined above and R<sub>1b</sub> is H, (c) R<sub>1b</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>- wherein R<sub>1b</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub> and A<sub>6</sub> are defined above, (d) R<sub>1b</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>- wherein R<sub>1b</sub>, A<sub>4</sub>, A<sub>5</sub> and A<sub>6</sub> are defined above, (e) R<sub>1b</sub>-A<sub>5</sub>-A<sub>6</sub>- wherein R<sub>1b</sub>, A<sub>5</sub> and A<sub>6</sub> are defined above, (f) R<sub>1b</sub>-A<sub>6</sub>- wherein R<sub>1b</sub> and A<sub>6</sub> are defined above and (g) R<sub>1b</sub>- wherein R<sub>1b</sub> is defined above; A<sub>7</sub> is Leu; A<sub>8</sub> is Leu or Nle; A<sub>9</sub> is His; A<sub>10</sub> is Asp or Asn; A<sub>11</sub> is Leu; A<sub>12</sub> is Gly; A<sub>13</sub>, A<sub>17</sub>, A<sub>18</sub>, A<sub>22</sub> and A<sub>30</sub> are independently selected from Ser, Thr, Lys, Cys, homo-Cys, Orn, Asp, Glu, -NHCH(CH<sub>2</sub>NH<sub>2</sub>)CO-, -NHCH[(CH<sub>2</sub>)<sub>2</sub>NH<sub>2</sub>]CO-, -NHCH[(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>H]CO- and -NHCH[(CH<sub>2</sub>)<sub>4</sub>CO<sub>2</sub>H]CO-; A<sub>14</sub> is His or Ser; A<sub>15</sub> is Ser or Leu; A<sub>16</sub> is Asn or Gln; A<sub>19</sub> is Arg or Glu; A<sub>20</sub> is Arg; A<sub>21</sub> is Arg or Val; A<sub>23</sub> is Phe or Trp; A<sub>24</sub> is Leu; A<sub>25</sub> is Arg or His; A<sub>26</sub> is His or is independently selected from the group consisting of Ser, Thr, Lys, Cys, homo-Cys, Orn, Asp, Glu, -NHCH(CH<sub>2</sub>NH<sub>2</sub>)CO-, -NHCH[(CH<sub>2</sub>)<sub>2</sub>NH<sub>2</sub>]CO-, -NHCH[(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>H]CO- and -NHCH[(CH<sub>2</sub>)<sub>4</sub>CO<sub>2</sub>H]CO-; A<sub>27</sub> is Leu or Lys; A<sub>28</sub> is Ile or Leu; A<sub>29</sub> is Ala or Gln; A<sub>31</sub> is Ile or Val; and Y is selected from the group consisting of (a) -R<sub>3</sub> wherein R<sub>3</sub> is -OH or -NR<sub>4</sub>R<sub>5</sub>, wherein R<sub>4</sub> and R<sub>5</sub> are independently selected from hydrogen and alkyl of one to four carbon atoms, (b) -A<sub>32</sub>-R<sub>3</sub> wherein R<sub>3</sub> is defined above and A<sub>32</sub> is His, (c) -A<sub>32</sub>-A<sub>33</sub>-R<sub>3</sub> wherein R<sub>3</sub> and A<sub>32</sub> are defined above and A<sub>33</sub> is Asn or Thr and (d) -A<sub>32</sub>-A<sub>33</sub>-A<sub>34</sub> wherein A<sub>34</sub> is Ala or Phe.

- 30 Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein

- (i) the side chains of A<sub>18</sub> and A<sub>22</sub> are linked through an amide bond,
- (ii) the side chains of A<sub>13</sub> and A<sub>17</sub> are linked through an amide bond, and the side chains of A<sub>18</sub> and A<sub>22</sub> are linked through an amide bond,
- (iii) the side chains of A<sub>18</sub> and A<sub>22</sub> are linked through an amide bond, and

- the side chains of A<sub>26</sub> and A<sub>30</sub> are linked through an amide bond or
- (iv) the side chains of A<sub>13</sub> and A<sub>17</sub> are linked through an amide bond,  
the side chains of A<sub>18</sub> and A<sub>22</sub> are linked through an amide bond and  
the side chains of A<sub>26</sub> and A<sub>30</sub> are linked through an amide bond.

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Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein A<sub>13</sub> is selected from Lys and Ala, A<sub>17</sub> is selected from Ser and Asp, A<sub>18</sub> is Lys, A<sub>22</sub> is Asp, A<sub>26</sub> is Lys and A<sub>30</sub> is Asp.

- 10 Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein X is R<sub>1a</sub>-A<sub>1</sub>-A<sub>2</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-.

Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein A<sub>1</sub> is Ala, A<sub>8</sub> is Nle and A<sub>27</sub> is Leu.

15

Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein R<sub>1a</sub> is H and Y is NH<sub>2</sub>.

- 20 Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein R<sub>1a</sub> is H and Y is -A<sub>32</sub>-A<sub>33</sub>-A<sub>34</sub>-NH<sub>2</sub>.

Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein X is selected from the group consisting of

- (a) R<sub>1b</sub>-A<sub>2</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-, (b) R<sub>1b</sub>-A<sub>3</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-, (c) R<sub>1b</sub>-A<sub>4</sub>-A<sub>5</sub>-A<sub>6</sub>-, (d) R<sub>1b</sub>-A<sub>5</sub>-A<sub>6</sub>-,  
25 (e) R<sub>1b</sub>A<sub>6</sub>- and (f) R<sub>1b</sub>-.

Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein A<sub>8</sub> is Nle and A<sub>27</sub> is Leu.

- 30 Another preferred cyclic peptide suitable for preparation using the process of this invention has formula I wherein X is H and Y is -A<sub>32</sub>-A<sub>33</sub>-A<sub>34</sub>-NH<sub>2</sub>.

More preferred cyclic peptides suitable for preparation using the process of this invention are selected from

- cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,2</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,3</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,4</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 5 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,5</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,6</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,7</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,10</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 10 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,11</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,12</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,13</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,14</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,15</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 15 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1,16</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 bicyclo(Lys<sup>13</sup>-Asp<sup>17</sup>,Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>17,22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 bicyclo(Lys<sup>18</sup>-Asp<sup>22</sup>,Lys<sup>26</sup>-Asp<sup>30</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>

Still more preferred cyclic peptides suitable for preparation using the process of this invention  
 20 are selected from

cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 bicyclo(Lys<sup>13</sup>-Asp<sup>17</sup>,Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>17,22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>  
 bicyclo(Lys<sup>18</sup>-Asp<sup>22</sup>,Lys<sup>26</sup>-Asp<sup>30</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>

25 A still yet more preferred cyclic peptide suitable for preparation using the process of this invention is  
 cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>

The foregoing may be better understood by reference to the following example, which is  
 30 presented for illustration and is not intended to limit the scope of this invention.

#### EXAMPLE 1

Preparation of cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>, Nle<sup>8</sup>, Lys<sup>18</sup>, Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>

Ala-Val-Ser-Glu-Ile-Gln-Leu-Nle-His-Asn-Leu-Gly-Lys-His-Leu-Asn-



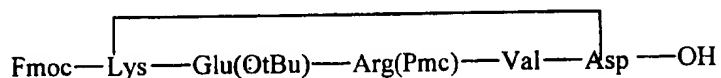
### Step 1:

Fmoc-Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin

5

Rink Amide MBHA Resin (0.5-0.7 meq./g titer, Nova Biochem, LaJolla, CA, USA), 5 g (0.64 mmole / g, 3.2 mmole) is swollen by shaking with 60 mL dimethylformamide, then the resin is drained and washed with two 60 mL portions of dimethyl formamide. The 9-fluorenylmethoxycarbonyl group (FMOC) is then removed by treating the resin with 60 mL of 20% piperidine / dimethylformamide for 20 minutes, followed by four 60 mL washes of the resin with dimethylformamide (TNBS: positive). Fmoc-Val-OH (2.17 g, 6.4 mmole) and 2.43 g (6.4 mmole) O-benzotriazole-1-yl-N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) in 150 mL dimethylformamide are treated with 32 mL (12.8 mmole) N-methylmorpholine at 0°C for 10 minutes, then the mixture is added to the resin peptide and the mixture agitated by shaking at ambient temperature for about 1.3 hour. The resin was drained, then washed with three 150 mL portions of dimethylformamide. (TNBS: negative). The resin was washed with two portions of diethyl ether and dried overnight; the weigh gain from the coupling was 0.88g, implying the addition of 2.75 mmole Val). The synthesis was continued by sequentially removing FMOC (as above) and coupling (as above) with 5.6 moles each of FMOC-Asp(OtBu), FMOC-Gln(Trt), FMOC-Leu, FMOC-Leu, FMOC-Lys(BOC), FMOC-Arg(PMC), FMOC-Leu, and FMOC-Trp(BOC), using 5.6 molar equivalents of HBTU, 11.2 mmole N-methylmorpholine, and 50 mL dimethylformamide in each coupling. The final weight gain was 5.8 g. An aliquot was cleaved to provide the title peptide retaining the FMOC but with side chains deprotected, of 86A% purity. LC-MS: 1390.6; calc. 1390.8).

25 Step 2: Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp.



Method A: Solid Phase Synthesis.

a. Sequence Assembly: Synthesis of Fmoc-Lys(Alloc)-Glu(OtBu)-Arg(Pmc)-Val-Asp(OAllyl)-Chlorotrityl resin.

Coupling of Fmoc-Asp(OAllyl)-OH to 15 g 1% crosslink chlorotrityl resin of 1.05 meq/g loading (total 15.75 mmole) was accomplished using 7.89 g (18.9 mmole) of Fmoc-Asp(OAllyl)-OH, 82 g (39.9 mmole) of benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP) and 10.2 g (78.8 mmole) of diisopropylethyl amine in 150 mL dichloromethane. The mixture was agitated by shaking, then drained and the resin washed using three 150 mL portions dichloromethane/methanol/diisopropylethylamine (17:2:1), followed by three 150 mL dichloromethane. The resin was sampled for a TNBS test, which was negative. The N-terminal 9-fluorenylmethoxycarbonyl group was removed by treating the Fmoc-protected resin-bound amino acid with 150 mL 5% piperidine in dichloromethane-dimethylformamide (1:1) for 10 minutes followed by 150 mL 20% piperidine in dimethylformamide for 30 minutes. The resin-bound amino acid was washed as described above. Fmoc-Val-OH, Fmoc-Arg(Pmc)-OH (10% aliquot removed after this step), Fmoc-Glu(OtBu)-OH and Fmoc-Lys( $\epsilon$ -Alloc)-OH are each coupled in the same fashion, and using the same sequence of events however using three to five dimethylformamide washes in place of three to dichloromethane washes before TNBS testing. No double couplings are required. The final resin bound peptide was washed with 150 mL tetrahydrofuran and 150 mL diethyl ether, then was dried under vacuum to afford 27.23 g

20

b. Allyl and Alloc Removal for Lys<sup>18</sup> and Asp<sup>22</sup>: Synthesis of Fmoc-Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-Chlorotrityl resin.

A 10 g portion of the Fmoc- (Lys-Alloc)-Glu(OtBu)-Arg(Pmc)-Val-Asp(OAllyl)-chlorotrityl resin prepared above was treated in a swollen in a shaker with 100 mL dimethylsulfoxide/dimethylformamide/dichloromethane (1:1:0.2 v/v/v) for 20 minutes, then the resin was gently drained. A partial solution of 2.9 g tetrakis(triphenylphosphine) palladium (0) in 240 mL dimethylsulfoxide/dimethylformamide (1:1, v:v) was added, followed by 60 mL dichloromethane and 16.2 g (150 mmole) N-methylalanine, then the mixture was agitated by shaking for 2 hours. The resin was drained and washed with 3 100 mL portions dichloromethane. The resin was then swollen with 100 mL dimethylsulfoxide/dimethylformamide (1:1 v:v), then gently drained and used directly in Lys<sup>18</sup>-Asp<sup>23</sup> cyclization.

30

c. Formation of Lys<sup>18</sup>-Asp<sup>22</sup> Lactam Bridge: Synthesis of Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-Chlorotrityl resin.

- 5 Resin Peptide from the above example is treated with 5.2 g (10 mmole) benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP) and 2.6 g (20 mmole) diisopropylethyl amine in 100 mL dimethylsulfoxide/dimethylformamide (1:1 v:v), and the mixture agitated for 4 hours. The resin was drained and washed with 3 100 mL portions of dimethylformamide, then an aliquot of drained resin was withdrawn for TNBS testing (negative). The resin was washed with 100 mL each of
- 10 tetrahydrofuran and diethyl ether, then was dried under vacuum.

d. Resin Cleavage and Product Isolation of Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp

- The resin peptide from the above Lys<sup>18</sup>-Asp<sup>22</sup> lactam bridge formation example was treated in a shaker
- 15 with 130 mL acetic acid/dichloromethane/2,2,2-trifluoroethanol (1:8:1) for 20 minutes. The liquid portion was drawn from the resin and treated with 500 mL methyl tert butyl ether and 100 mL diethyl ether, and the mixture centrifuged. The supernatant was removed, and to the residue was added diethyl ether, followed by centrifugation. The supernatant was removed and the residue was dried overnight to afford 2.77 g of a yellow solid (MS: 1172; 41% stoich. yield, crude). This was purified by flash
- 20 chromatography (85:15 to 60:40 dichloromethane : methanol) to afford 1 g Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-OH (15 % yield based on resin) with 85A% purity.

Method B: Synthesis in Solution.

- 25 a. Sequence Assembly

Synthesis of BOC-Asp(OAllyl)-OBzl

- In a 3 neck, 3 L flask charged with 1,1-dimethylethoxycarbonyl-(L)-aspartic acid benzyl ester
- 30 (50 g, 154.5 mmol), dichloromethane (96mL), allyl alcohol (9.43 g, 162.3 mmol) and 4-N,N-dimethylamino pyridine (10 mg) at ambient temperature. The reaction mixture is cooled to 8 °C, and a solution of dicyclohexylcarbodiimide (32 g, 154.5 mmol) in dichloromethane (100 mL) is added over 1.5 hours at 8-11 °C. As th dicyclohexylcarbodiimide solution is added, dicyclohexyl urea precipitates. The reaction mixture is stirred for 2 hours at 11-12 °C, at which point HPLC shows no

starting material. The dicyclohexyl urea is filtered off and the filtrate is concentrated. The oily residue is taken up in methyl-t-butyl ether (250 mL), and the solution is filtered to remove residual dicyclohexyl urea, washed with 30 mL 0.2N hydrochloric acid, 30 mL water, 20 mL saturated aqueous sodium bicarbonate, 30 mL water, and two 50 mL portions of brine, dried (magnesium sulfate), filtered, and  
5 concentrated to give 55.88 g of BOC-(L)Asp(OAllyl)-OBzl as an oil. ( $M_{\text{calc}}$  363.4;  $M+1_{\text{obsd}}$  364).

#### Synthesis of HCl-Asp(OAllyl)-OBzl

A solution of BOC-Asp(OAllyl)-OBzl (110.5 g, 302.7 mmol) in 500 mL ethyl acetate in a 3  
10 neck, 3L flask with a mechanical stirrer is cooled to 5 °C and hydrogen chloride gas is bubbled through the reaction mixture for 2.5 hours during which time the reaction temperature reaches about 22 °C. HPLC shows no starting material. Nitrogen is bubbled into the solution overnight, resulting in formation of a white precipitate which is collected by filtration and washed with 100 mL of ethyl acetate. The residue is dried under vacuum to afford 84 g HCl•Asp(OAllyl)-OBzl as a white crystalline (microscopy)  
15 solid. ( $M_{\text{calc}}$  263;  $M+1_{\text{obsd}}$  264).

#### Synthesis of BOC-Val-Asp(OAllyl)-OBzl

To a solution of BOC-Val-OH (25.4g, 116.7 mmol) in ethyl acetate (85 mL) is added  
20 hydroxybenzotriazole hydrate (16.55g, 122.5mmol) in dimethylformamide (122.3 mL). The mixture is cooled to 5°C and a solution of dicyclohexylcarbodiimide (25.2 g, 122.5 mmol) in ethyl acetate (58.7 mL) is added over 1 hour during which time the temperature rises to about 18 °C and dicyclohexyl urea precipitates. Cooling is ceased and the mixture is stirred for 2 hours and a solution of HCl•H<sub>2</sub>N-Asp(OAllyl)-OBzl (35 g, 116.7 mmol) in 189 mL of dimethylformamide is added over 25 minutes. N-  
25 methylmorpholine (12.8 mL) is added slowly and over 0.5 hours to attain a reaction mixture pH of 7 and the mixture is stirred at ambient temperature for 36 hours. The reaction mixture is filtered to remove dicyclohexyl urea, the filtrate is diluted with methyl-t-butyl ether, stirred for 2 hours, then refiltered. The  
filtrate is washed with 100 mL water, 100 mL 0.5M aqueous citric acid, 100 mL water, 100 mL saturated  
aqueous, sodium bicarbonate, 100 mL brine, dried (magnesium sulfate), filtered, and concentrated to  
30 give 41.3 g of BOC-Val-Asp(OAllyl)-OBzl as an oil. ( $M_{\text{calc}}$  462;  $M+1_{\text{obsd}}$  463).

#### Synthesis of HCl•Val-Asp(OAllyl)-OBzl

A 2-4°C solution of BOC-Val-Asp(OAll)-OBzl (31.7g, 68.5 mmol) in ethyl acetate (300 mL) is charged with hydrogen chloride gas for 2 hours. Cooling is ceased and the mixture is stirred for 48 hours. Nitrogen is rigorously bubbled into the solution for 1 hour. The reaction mixture is concentrated on the rotary evaporator to give 38.3 g of a white solid, which is crystallized from ethyl acetate to afford  
5 24.2 g of HCl•Val-Asp(OAllyl)-OBzl. ( $M_{\text{calc}}$  362  $M+1_{\text{obsd}}$  363).

#### Synthesis of Fmoc-Arg(PMC)-Val-Asp(OAllyl)-OBzl

In a 3 neck, 3 L flask equipped with a mechanical stirrer is placed 17.25 g (26 mmol) Fmoc-Arg(PMC) and 300 mL acetonitrile and the mixture is stirred at ambient temperature until a clear  
10 solution is obtained. Benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP) is added, the solution cooled to 2 °C, and 2.6 g (26 mmol) of N-methylmorpholine is added, forming a white tar. Dimethylformamide (52 mL) is added slowly until the tar dissolves, and the mixture is stirred at 3 °C for 30 minutes. A solution of HCl•Val-Asp(OAllyl)-OBzl (10.4 g, 26 mmol) in 39 mL of  
15 dimethylformamide is added over 20 minutes at 2-3 °C, followed by N-methylmorpholine (7.5 mL) to attain pH 7. Cooling is ceased, and the mixture is stirred for 2.5 hours. The mixture is then diluted with 250 mL water and 250 mL ethyl acetate. The ethyl acetate layer is removed and washed with water (2 x 100 mL) and brine (2 x 100 mL) and concentrated to afford a white solid which is crystallized from 175 mL ethyl acetate to give 18.5 g Fmoc-Arg(PMC)-Val-Asp(OAllyl)-OBzl (95A%). The ethyl acetate  
20 filtrate is concentrated and the gummy residue triturated with ethyl acetate to afford an additional 3.12 g of product. ( $M_{\text{calc}}$  1007;  $M+1_{\text{obsd}}$  1008).

#### Synthesis of Arg(PMC)-Val-Asp(OAllyl)-OBzl

To a solution of Fmoc-Arg(PMC)-Val-Asp(OAllyl)-OBzl (16 g, 15.9 mmol) in  
25 dichloromethane (267 mL) is added piperidine (23 g, 27 mmol, 26.7 mL) at ambient temperature. The reaction is stirred 2.5 hours and then is washed with water (2 x 60 mL) and brine (2 x 60 mL), dried (magnesium sulfate), filtered, and concentrated to give a semi-solid. The semi-solid is taken up in 400  
30 mL ethyl acetate and the solution is left standing for three days and then is filtered. The filtrate is washed with water (2 x 60 mL), dried (magnesium sulfate), filtered and concentrated to afford 23.5 g of product contaminated with piperidine-benzofulvene adduct (MS analysis). The solid is dissolved in 400 mL ethyl acetate, filtered, washed with water (2 x 60 mL), dried (magnesium sulfate), and concentrated to afford a solid which is dried overnight under vacuum to afford 12.55 g of Arg(PMC)-Val-Asp(OAllyl)-OBzl as a white solid. ( $M_{\text{calc}}$  784  $M+1_{\text{obsd}}$  785).

## Synthesis of Fmoc-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-OBzl

To a solution of Fmoc-Glu(tBu) (6.73 g, 15.8 mmol) and benzotriazolyloxy-tris[pyrrolidino]-  
5 phosphonium hexafluoroacetate (PyBOP, 8.18 g, 15.8 mmol) in 105 mL acetonitrile at 2 °C is added N-  
methylmorpholine (1.58 g, 15.8 mmol) and the reaction mixture is stirred at 2-4 °C for 30 minutes. A  
solution of L)Arg(PMC)-Val-Asp(OAllyl)-OBzl (12.4 g, 15.8 mmole) in 70 mL acetonitrile is added at 2  
°C over 20 minutes. N-methylmorpholine is then added to achieve pH 7. Cooling is ceased, and the  
mixture is allowed to warm to ambient temperature, then stirred is continued for a total of 3 hours. The  
10 milky reaction mixture is filtered (slow) and the white residue is dried in the filter overnight to afford  
10.8 g of Fmoc-Glu(OBut)-Arg(PMC)-Val-Asp(Allyl)-OBzl. The filtrate is concentrated to give a  
gum, which is taken up in 30 mL acetonitrile and allowed to stand for 48 hours, producing an additional  
3.45 g of product. The total yield of Fmoc-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-OBzl is 14.3 g.  
( $M_{calc}$  1192;  $M+1_{obsd}$  1192.4 )

15

## Synthesis of Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-OBzl

To a solution of Fmoc-Glu(OBut)-Arg(PMC)-Val-Asp(OAllyl)-OBzl (12.3g, 10.3 mmol) in 105  
mL of dichloromethane is added piperidine (3.5g, 41.3 mmol) at ambient temperature. The mixture is  
20 stirred 2 hours, then diluted with heptane, concentrated to half volume, and left standing in the freezer  
overnight. The liquor is decanted from the oily residue, which is then taken up in dichloromethane. The  
solution is washed with 0.5M aqueous citric acid, water, and brine, dried (magnesium sulfate), filtered,  
and concentrated. The crude solid is triturated twice with heptane and filtered to give 9.0 g of  
Glu(OtBu)-(L)Arg(PMC)-Val-Asp(OAllyl)-OBzl as a white powder. ( $M_{calc}$  970.2;  $M+1_{obsd}$  971).

25

## Synthesis of Fmoc-Lys(Alloc)-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-OBzl

A suspension of Fmoc-Lys(Alloc) (4.2 g, 9.3 mmol) and benzotriazolyloxy-tris[pyrrolidino]-  
phosphonium hexafluoroacetate (PyBOP, 4.8 g, 9.3 mmol) in 69 mL acetonitrile at 5 °C is treated with  
30 N-methylmorpholine (0.94 g, 9.3 mmol), resulting in formation of a solution which is stirred at 5 °C for  
30 minutes. A suspension of Glu(OtBu)-Arg(PMC)-Val-Asp(Allyl)-OBzl (9 g, 9.3 mmole) in 52 mL  
acetonitrile is added, causing the reaction mixture to become cloudy white. N-methylmorpholine is  
added until the reaction mixture is at pH 7, then the mixture is allowed to warm to ambient temperature  
and stir for 1.33 hours. The mixture is filtered and the solid residue is washed with acetonitrile (2 x 40

mL) and dried overnight under vacuum with a nitrogen bleed to give 12.8 g of an off-white crusty solid which is triturated with 200 mL methyl-t-butyl ether, collected by filtration and dried under vacuum to afford 9.7 g of Fmoc-Lys(Alloc)-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-OBzl. ( $M_{\text{calc}}$  1404.7;  $M+1_{\text{obsd}}$  1406).

5

b. Alloc and Allyl removal From Lys<sup>18</sup> and Asp<sup>22</sup>: Synthesis of Fmoc-Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OBzl

10 A 3 neck 1L flask equipped with mechanical stirrer and nitrogen flow is charged at ambient temperature with 167 mL of dichloromethane and 1.37 g (1.18 mmol, 0.2 eq.) of Pd(PPh<sub>3</sub>)<sub>4</sub>. Nitrogen is bubbled into the orange solution for 2 minutes, then a solution of 8.3 g, (5.91 mmol, 1.0 eq.) of Fmoc-Lys(Alloc)-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-OBzl in 66.4 mL of dimethylformamide is added, followed by 13.3 g, (124 mmol, 21 eq.) of methylaniline. The solution is stirred at ambient temperature for 1 hour. The mixture is diluted with 700 mL of methyl-t-butyl ether which causes a solid to form. The  
15 suspension is stirred for 1 hour and filtered. The solid residue is triturated with 200 mL of methyl-t-butyl ether, filtered and dried to obtain 5.0 g of a pale yellow solid. The filtrate yields 1.1 g of a second crop as a pale yellow solid for a total yield of 6.1 g of Fmoc-Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OBzl. ( $M_{\text{calc}}$  1280.6;  $M+1_{\text{obsd}}$  1281).

20 c. Lys<sup>18</sup>-Asp<sup>22</sup> Lactam Bridge Formation: Synthesis of Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OBzl

In a 3 neck 1L flask is dissolved 6.0 g, (4.69 mmol) of Fmoc-Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OBzl in 300 mL of dimethyl formamide at room temperature under nitrogen and 3.44 g, (6.61  
25 mmol, 1.41 eq.) of benzotriazolyl-oxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP), 0.89 g, (6.57 mmol, 1.40 eq.) of hydroxybenzotriazole hydrate, and 1.73 g, (13.4 mmol; 2.85 eq.) of diisopropylethyl amine are added. The orange solution is stirred for 1 hour at ambient temperature, then  
is diluted with 300 mL of water which results in formation of an oily residue. The cloudy liquor (contain  
mostly impurities) is decanted. The oily residue is stirred overnight with 100 mL of water, affording a  
30 crusty solid which is collected by filtration, giving 4.2 g of a pale yellow solid. The filtrate affords a 1.0 g of a second crop as a yellow solid, for a total yield of 5.2 g of Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OBzl. ( $M_{\text{calc}}$  1262  $M+1_{\text{obsd}}$  1262.5).

d. Removal of Benzyl Ester: Synthesis Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OH

A solution of 5.9 g (4.7 mmol) of Fmoc-cyclo(Lys-Asp)Lys-Glu-Arg-Val-Asp-OBzl in 25 mL dimethylformamide is treated with 5.9 g charcoal and filtered, and then is treated with 0.59 g 10% Pd/C and placed under 50 psig of hydrogen on a Parr apparatus. After 1.5 hours, the mixture is filtered, treated again with charcoal, refiltered, and recharged with 0.59 g 10% Pd/C and exposed to 50 psig hydrogen for an additional 2 hours. The mixture is filtered and the filtrate is diluted with 50 mL water and extracted with three 200 mL portions of methylene chloride. The combined methylene chloride extracts are concentrated to afford 1.2 g of Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OH an oil. ( $M_{\text{calc}}$  1171;  $M+1_{\text{obsd}}$  1172.3).

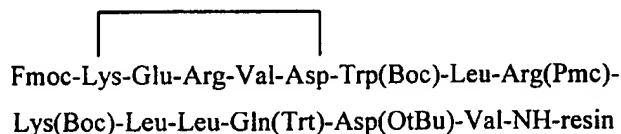
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### 3. Purification

A 400 mg sample of Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OH is dissolved in 10 mL 1:1 trifluoroethanol/isopropanol. The sample is injected in two 5 mL slugs onto a preparative HPLC column (Microsorb MV C18, 300Ang, 8u, 41.4 X 250 mm) equilibrated with 55% B (A = 0.1% v/v aqueous trifluoroacetic acid; B = v/v 75% acetonitrile/25% isopropanol with 0.1% trifluoroacetic acid). The charge is eluted at 70 mL / minute in a gradient of 55% B to 65% B over 30 minutes, 65%B to 95% B over 1 minute, followed by isocratic column washing with 95%B for 10 minutes. Effluent is monitored using UV at 210nm. Fractions with purity of 93.5% or higher are pooled and concentrated by rotary evaporation. Lyophilization of the residue affords 200 mg of Fmoc-cyclo(Lys-Asp)Lys-Glu-Arg-Val-Asp-OH ( $M_{\text{calc}}$  1171;  $M+1_{\text{obsd}}$  1172.6).

20

#### Step 3:



25

FMOC-[Trp<sup>23</sup>(BOC), Arg<sup>25</sup>(PMC), Lys<sup>26</sup>(BOC), Leu<sup>27</sup>, Gln<sup>29</sup>(Trt)]HPHT(23-31)-chlorotrityl resin (1 g, 0.185 mmole) was swollen in 10 mL dimethylformamide with shaking agitation for 30 minutes, then was drained and treated with 10 mL 20% piperidine/dimethylformamide (v/v) with shaking agitation for 30 minutes. The resin was drained and washed with 3 20 mL portions of dimethylformamide. A solution of 240 mg (0.205 mmole, 1.1 eq.) of Fmoc-cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OH, 312 mg (0.6 mmole, 3 eq.) benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP), and 156 mg (1.2 mmole, 6 eq) diisopropylethyl amine in 10 mL dimethylformamide was added and the

30

mixture agitated for 16 h. The resin was drained and washed with three 20 mL portions of dimethylformamide (TNBS positive) and retreated with of Fmoc-cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-OH/PyBOP/iPr<sub>2</sub>NEt a second time for 16 h, then the resin was drained and washed with three 10 mL portions dimethylformamide (TNBS: negative). An aliquot of the title peptide was  
 5 cleaved from the resin as described in Step 2, Method A using Reagent K, to afford the side-chain deprotected peptide fragment. (LC-MS: M<sub>calc</sub> 2001; M<sub>obsd</sub> 2000.5). The remainder was used in Step 4.

Step 4:

Fmoc-Lys(Boc)-His(Trt)-Leu-Asn(Trt)-Ser(OtBu)-Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-  
 Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin

10

The desired resin-bound peptide was prepared by removal of the Fmoc protecting group from 1.38 g (0.244 mmole based upon titer at Trp<sup>23</sup>) of the resin-bound peptide of Step 3 as per Step 1, followed by sequential cycling of Fmoc removal and coupling with 0.5 mmole each of Fmoc-Ser(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, and Fmoc-Lys(BOC)-OH  
 15 as per Step 1, using 190 mg (0.5 mmole) of O-benzotriazole-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and 2 mmole) of N-methylmorpholine in 25 mL dimethylformamide. The couplings were performed at ambient temperature over about 1 hour. The final resin peptide was washed with 25 mL portions of dimethylformamide, tetrahydrofuran, and diethyl ether and dried under vacuum (1.93 g) An aliquot of the title peptide was cleaved from the resin as described below (Reagent K), to  
 20 afford the side-chain deprotected peptide fragment (LC-MS: M<sub>calc</sub> 2579.4; M<sub>obsd</sub> 2580.7) of 52A% purity.

Step 5:

Fmoc-Ala-Val-Ser(OtBu)-Glu(OtBu)-Ile-Gln(Trt)-Leu-Nle-His(Trt)-Asn(Trt)-Leu-Gly-OH  
 25 Fmoc-[Ala<sup>1</sup>, Ser<sup>3</sup>(OtBu), Glu<sup>4</sup>(OtBu), Gln<sup>6</sup>(Trt), His<sup>9</sup>(Trt), Asn<sup>10</sup>(Trt)Nle<sup>8</sup>]hPTH(1-12)

The title peptide is prepared by coupling 2.65 g (5 mmole) Fmoc-Leu-OH to 5 g (3 mmole) of 1% crosslink Gly-2-chlorotriptyl resin of 0.6 meq/g with 3.9 g (7.5 mmole) benzotriazoloxyl-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP), 1.94 g (15 mmole) diisopropyl ethyl amine in  
 30 50 mL dimethylformamide / tetrahydrofuran (v/v) using shaking agitation, for 0.5 hours. The resin was drained, then washed with five portions of dimethylformamide / tetrahydrofuran and one portion of methanol (Ninhydrin: negative). The resin was washed once with dimethylformamide, then treated twice with 60 mL 20% piperidine / dimethylformamide for 15 minutes each time, with three 60 mL portions

of dimethylformamide and one 60 mL portion of methanol between each treatment. After the second such treatment, the ninhydrin was highly positive. This cycle was repeated, using a single 0.5 hour piperidine dimethylformamide treatment in each subsequent case, and using 2.5 molar equivalents each of Fmoc-Asn(Trt), Fmoc-His(Trt), Fmoc-Nle, Fmoc-Leu, Fmoc-Gln(Trt), Fmoc-Ile, Fmoc, Glu(OtBu), Fmoc-Ser(OtBu), Fmoc-Val, and Fmoc-Ala, along with 2.5 molar equivalents each of PyBOP and diisopropylethyl amine. The resin peptide was cleaved by shaking with 100 mL acetic acid / trifluoroethylene / and dichloromethane (1 : 1 : 8) for 1 hour. The liquid was drained from the resin, and the resin was washed with 200 mL dichloromethane; then wash was combined with the main portion of the liquid. This was evaporated sequentially from hexane, ethyl acetate, and isopropanol. To the residue was triturated with diethyl ether, then dried (overnight, vacuum) to afford 2.24 g of the title peptide (HPLC: 83A% pure). The resin was retreated under cleavage conditions a second time and the liquid treated as after the first cleavage, to finally afford 2.75 g of a second crop of the title peptide (HPLC: 00A% pure), for a total yield of 4.95 g (2.1 mmole, 70% yld. from resin). [LC-MS ( $M_{calc}$  1514.8;  $M_{obsd}$  1514.7)]. This material was used as obtained in Step 6.

15

Step 6:

Fmoc-Ala-Val-Ser(OtBu)-Glu(OtBu)-Ile-Gln(Trt)-Leu-Nle-His(Trt)-Asn(Trt)-Leu-Gly-  
 Lys(Boc)-His(Trt)-Leu-Asn(Trt)-Ser(OtBu)-Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-  
 Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin

20 The peptide prepared in Step 5 (900 mg, 0.114 mmole) was swollen in 15 mL dimethylformamide for 40 minutes with shaking agitation, then the resin was drained and treated with 15 mL 20% piperidine/dimethylformamide (v/v) for 45 minutes. The resin was drained was washed with three 30 mL portions dimethylformamide (resin TNBS test: positive). To this was added 15 mL dimethylformamide, 775 mg, (0.33 mmole, 2.7 molar eq. based upon titer at position 23)) Fmoc-[Ala<sup>1</sup>, Ser<sup>2</sup>(OtBu), Glu<sup>4</sup>(OtBu), Gln<sup>6</sup>(Trt), His<sup>9</sup>(Trt), Asn<sup>10</sup>(Trt)Nle<sup>8</sup>]hPTH(1-12) (from Step 4), 151 mg (0.29 mmole, 0.21 molar eq.) benzotriazolyl-oxy-tris[pyrrolidino]-phosphonium hexafluoroacetate (PyBOP), 39 mg (0.25 mole) hydroxybenzotriazole hydrate and 75 mg (0.58 mmole) diisopropylethyl amine, and the mixture agitated by shaking for 3 days. The resin was drained, washed with three 30 mL portions of dimethylformamide, (TNBS, mix of sl. positive and negative beads), then treated with 15 mL 20% piperidine/dimethylformamide (v/v), drained, and washed with three 15 mL portions each of dimethylformamide, tetrahydrofuran, and diethyl ether, then was dried and deprotected and cleaved from resin as per Step 7.

Step 7:

Ala-Val-Ser-Glu-Ile-Gln-Leu-Nle-His-Asn-Leu-Gly-Lys-His-Leu-Asn-



- 5 The resin peptide from Step 6 was treated to remove Fmoc on Ala<sup>1</sup> as per Step 1 Method A, then simultaneously cleaved and deprotected in a 33/2/2/1/2 mixture of trifluoroacetic acid, phenol, thioanisole, ethanedithiol, and water (Reagent K total dilution 10 mL/g resin-peptide) for 3 hours. The resin was drained and product was precipitated from the filtrate by adding the reaction mixture to cold t-butylmethyl ether (50 mL/g resin-peptide). The mixture was left standing for 15 minutes, then was
- 10 centrifuged (2000 rpm, 5 minutes). The liquid was decanted and the remaining solid was washed with diethyl ether, the mixture supernatant was separated by centrifugation and the supernatant removed. The resulting solid is stirred in 0.1% aqueous trifluoroacetic acid (6 mL / g resin peptide) for 1 hour to ensure complete removal of acid sensitive groups. The solution is lyophilized to afford 414 mg of the title peptide (37A% pure, 26.3% pure by wt/wt, 26.3% yld from Trp<sup>23</sup>) as a white solid. (LC-MS: M<sub>calc</sub>
- 15 3632.0; M+1<sub>obsd</sub> 3633.4).

Step 8: Purification

- 20 One gram of the material prepared above is dissolved in 10 mL water and the solution is injected onto a 2" X 25 cm preparative HPLC (10 micron, 120A or 300A) pre-equilibrated with 75/5/20 0.1% aq. trifluoroacetic (A) / 0.1% trifluoroacetic acid in acetonitrile (B) / isopropanol (C). The column is eluted at 115 mL per minute with a gradient 75A/5B/20C to 60A/20B/20C over 30 minutes, then flushed with 30%A, 60%B, 20%C and re-equilibrated with 75A/5B/20C. Effluent is monitored using UV detection at 280nm. Fractions containing the title peptide of ca. 75 area percent purity are combined and
- 25 concentrated on a rotary evaporator (~35°C). The concentrated aqueous solution is pumped onto the same preparative column and rerun through the same 30 minute gradient. Fractions containing the title peptide at 90A% or greater area percent purity are combined and concentrated on a rotary evaporator at (~35°C). The concentrated solution is then pumped onto the preparative column which had been pre-equilibrated with 75% 0.1M aq. ammonium acetate (adjusted to pH 6 with acetic acid) (D) / 15%
- 30 acetonitrile (E) / 10% isopropanol (C). The column is eluted at 115 mL / minute with 75D/15E/10C to 60D/30E/10C over 30 minutes, then flushed with 30D/60E/20C. Fractions containing the title peptide of 95A% or higher percent area purity are combined and lyophilized, providing purified product as the

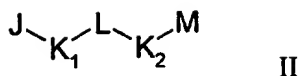
solid, acetate salt (1 g crude gives ca. 100-200 mg @ ca. 95A% purity). At each stage, fractions are analyzed for content and purity using analytical HPLC on a C18 column (4.6 mm x 25cm, 5 micron, 120A or 300A) in a gradient of 25-40%B (A = 0.1% Trifluoroacetic acid / water; B = 0.1% trifluoroacetic acid / acetonitrile) over 30 minutes using a flow of 1 mL / minute and UV monitoring at

5 220 nm.

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## CLAIMS:

1. A method for preparing:



wherein

J, L, and M are linear peptide fragments

K<sub>1</sub> is absent or a cyclic peptide fragment, and

K<sub>2</sub> is a cyclic peptide fragment;

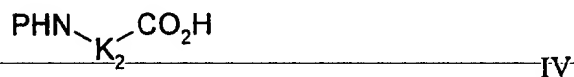
this method comprising the steps of:

(1) preparing  $\text{M}-\text{R}$  by sequential attachment of suitably protected amino acid residues to a resin to provide:



where  $\text{R}$  is a suitable peptide synthesis resin and M is a polypeptide fragment;

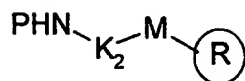
(2) preparing separately by conventional peptide synthesis N-terminal protected cyclic polypeptide fragment of formula IV



wherein P is a suitable amine protecting group,

(3) Coupling III with IV to provide a peptide of formula V

45

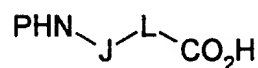


V;

(4) if  $\text{K}_2$  is absent, then (a) preparing polypeptide fragments J and L as a single polypeptide of formula

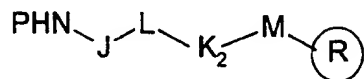
VI

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VI

and coupling polypeptide VI to peptide V to provide a peptide of formula VII



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VII

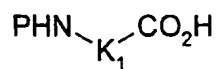
or, optionally, (b) adding the protected individual amino acid elements of polypeptides J and L sequentially to the peptide fragment of formula V,

15 or, optionally, (c) separately preparing fragments either or both J and L as polypeptide fragments and coupling these fragments to the growing peptide starting with the fragment of formula V;

(5) when cyclic peptide  $\text{K}_1$  is present, then (a) preparing separately polypeptide fragment of formula

VIII

20



VIII

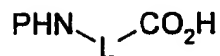
wherein

P is a suitable amine protecting group,

25

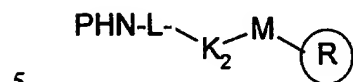
by conventional peptide synthesis procedures;

(b) preparing a peptide fragment of formula IX



IX

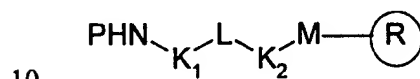
and coupling this fragment with peptide fragment V to provide a peptide fragment of formula X



5

X;

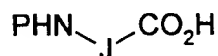
and (c) coupling the cyclic peptide fragment VIII with the peptide fragment of formula X to provide a peptide fragment of formula XI



10

XI;

(d) preparing peptide fragment of formula XII



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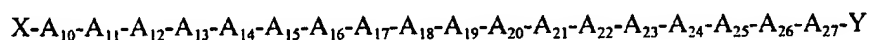
XII

and coupling fragment XII with fragment XI; and

(6) cleaving the resin and deprotecting.

20

2. The method of Claim 1, wherein the peptide of formula I is a cyclic peptide compound of the formula



25

wherein

X is selected from the group consisting of

- (a)  $\text{R}_{1a}-\text{A}_0-\text{A}_1-\text{A}_2-\text{A}_3-\text{A}_4-\text{A}_5-\text{A}_6-\text{A}_7-\text{A}_8-\text{A}_9-$ ,  
 (b)  $\text{R}_{1a}-\text{A}_2-\text{A}_3-\text{A}_4-\text{A}_5-\text{A}_6-\text{A}_7-\text{A}_8-\text{A}_9-$ ,  
 (c)  $\text{R}_{1b}-\text{A}_3-\text{A}_4-\text{A}_5-\text{A}_6-\text{A}_7-\text{A}_8-\text{A}_9-$ ,  
 (d)  $\text{R}_{1a}-\text{A}_4-\text{A}_5-\text{A}_6-\text{A}_7-\text{A}_8-\text{A}_9-$ ,  
 (e)  $\text{R}_{1a}-\text{A}_5-\text{A}_6-\text{A}_7-\text{A}_8-\text{A}_9-$ ,  
 (f)  $\text{R}_{1a}-\text{A}_6-\text{A}_7-\text{A}_8-\text{A}_9-$ ,

30

- (f)  $R_{1a}-A_6-A_7-A_8-A_9$ ,  
 (g)  $R_{1a}-A_7-A_8-A_9$ ,  
 (h)  $R_{1a}-A_8-A_9$ ,  
 (i)  $R_{1a}-A_9$ , and  
 5 (j)  $R_{1a}$ ;

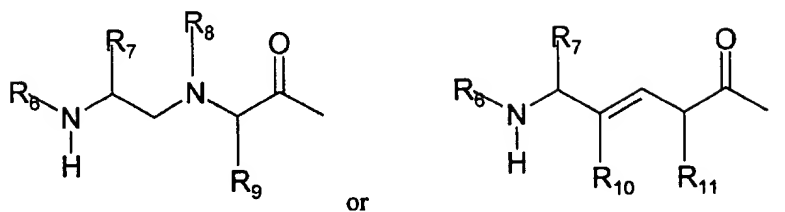
Y is selected from the group consisting of

- (a)  $-R_3$ ,  
 (b)  $-A_{28}-R_3$ ,  
 10 (c)  $-A_{28}-A_{29}-R_3$ ,  
 (d)  $-A_{28}-A_{29}-A_{30}-R_3$ ,  
 (e)  $-A_{28}-A_{29}-A_{30}-A_{31}-R_3$ ,  
 (f)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-R_3$ ,  
 (g)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-A_{33}-R_3$ , and  
 15 (h)  $-A_{28}-A_{29}-A_{30}-A_{31}-A_{32}-A_{33}-A_{34}-R_3$ ;

$R_{1a}$  is H, alkyl, aralkyl or  $-\text{COR}_2$ ;

$R_{1b}$  is  $R_{1a}$  or a group of formula

20



$R_2$  is alkyl, alkenyl, alkynyl, aryl or aralkyl;

25  $R_3$  is a group of formula  $A_{35}-\text{OR}_4$  or  $A_{35}-\text{NR}_4\text{R}_5$ ;

$R_4$  and  $R_5$  are independently H or lower alkyl;

$R_6$  and  $R_9$  are independently H or alkyl;

30

R<sub>7</sub> is alkyl;

R<sub>8</sub> is H, alkyl or COR<sub>2</sub>;

5 R<sub>10</sub> is H or halogen;

R<sub>11</sub> is alkyl or aralkyl;

m is 1, 2 or 3;

10

n is 3 or 4;

A<sub>0</sub> is absent or a peptide of from one to six amino acid residues;

15 A<sub>1</sub> is Ser, Ala, Gly or D-Pro, or an equivalent amino acid;

A<sub>2</sub> is Ala, Val or Gly, or an equivalent amino acid;

A<sub>3</sub> is Ala, Ser, Gly or D-Pro, or an equivalent amino acid;

20

A<sub>4</sub> is Glu, Ala or Gly, or an equivalent amino acid;

A<sub>5</sub> is Ile, His, Ala or Gly, or an equivalent amino acid thereof;

25 A<sub>6</sub> is Ala, Gln, Gly or D-Pro, or an equivalent amino acid thereof;

A<sub>7</sub> is Ala, Leu, Gly, or an equivalent amino acid thereof;

---

A<sub>8</sub> is Leu, Nle, Gly or D-Pro, or an equivalent amino acid thereof;

30

A<sub>9</sub> is His, Ala, D-Pro or Gly, or an equivalent amino acid thereof;

A<sub>10</sub> is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, D-Pro,  
-NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>11</sub> is Ala, Gly, Leu or Lys, or an equivalent amino acid thereof;

A<sub>12</sub> is Ala or Gly, or an equivalent amino acid thereof;

5

A<sub>13</sub> is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

10 A<sub>14</sub> is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, His, Lys, Orn, Ser, Thr, D-Pro, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>15</sub> is Ala, Gly, Ile, D-Pro or Leu, or an equivalent amino acid thereof;

A<sub>16</sub> is Asn, Ala, Gly, D-Pro or Gln, or an equivalent amino acid thereof;

15

A<sub>17</sub> is Ala, Asn, Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr, D-Pro, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

20 A<sub>18</sub> is Asp, Cys, homo-Cys, Glu, His, Leu, Lys, Orn, Nle, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>19</sub> is Arg or Glu, or an equivalent amino acid thereof;

A<sub>20</sub> is Arg or an equivalent amino acid thereof;

25

A<sub>21</sub> is Arg, Asp, Cys, homo-Cys, Glu, Lys, Orn, Ser, Thr, Val, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

30 A<sub>22</sub> is Asp, Cys, homo-Cys, Glu, His, Lys, Orn, Phe, Ser, Thr, -NHCH(CH<sub>2</sub>)<sub>m</sub>NH<sub>2</sub>)CO- or -NHCH[(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>H]CO-;

A<sub>23</sub> is Leu, Phe or Trp, or an equivalent amino acid thereof;

A<sub>24</sub> is Leu or an equivalent amino acid thereof;

$A_{25}$  is Arg, Asp, Cys, homo-Cys, Glu, His, Lys, Orn, D-Pro, Ser, Thr,  $-NHCH(CH_2)_mNH_2CO-$  or  $-NHCH[(CH_2)_nCO_2H]CO-$ ;

5  $A_{26}$  is Asp, Cys, homo-Cys, Glu, His, Lys, Orn, Ser, Thr,  $-NHCH(CH_2)_mNH_2CO-$  or  $-NHCH[(CH_2)_nCO_2H]CO-$ ;

$A_{27}$  is Leu or Lys, or an equivalent amino acid thereof;

10  $A_{28}$  is Ile or Leu, or an equivalent amino acid thereof;

$A_{29}$  is Ala, Asp, Cys, homo-Cys, Glu, Gln, Lys, Orn, Ser, Thr,  $-NHCH(CH_2)_mNH_2CO-$  or  $-NHCH[(CH_2)_nCO_2H]CO-$ ;

15  $A_{30}$  is Asp, Cys, homo-Cys, Glu, Gly, Lys, Orn, Ser, Thr,  $-NHCH(CH_2)_mNH_2CO-$  or  $-NHCH[(CH_2)_nCO_2H]CO-$ ;

$A_{31}$  is Ile, Leu or Val, or an equivalent amino acid thereof;

20  $A_{32}$  is His, or an equivalent amino acid thereof;

$A_{33}$  is Asn or Thr, or an equivalent amino acid thereof; and

$A_{34}$  is Ala or Phe, or an equivalent amino acid thereof;

25

$A_{35}$  is absent or a peptide of from 1 to 4 amino acids; and

---

the side chains of at least one of the following pairs of amino acid residues,  $A_{10}$  and  $A_{14}$ ,  $A_{13}$  and  $A_{17}$ ,  $A_{14}$  and  $A_{18}$ ,  $A_{17}$  and  $A_{21}$ ,  $A_{18}$  and  $A_{22}$ ,  $A_{21}$  and  $A_{25}$ ,  $A_{25}$  and  $A_{29}$  and  $A_{26}$  and  $A_{30}$  are linked through an amide, ester, disulfide or lanthionine bond to form a bridge, and the side chain of each of the following amino acid residues,  $A_{10}$ ,  $A_{13}$ ,  $A_{14}$ ,  $A_{17}$ ,  $A_{18}$ ,  $A_{21}$ ,  $A_{22}$ ,  $A_{25}$ ,  $A_{26}$ ,  $A_{29}$ , and  $A_{30}$  contributes, at most, to the formation of a single bridge; provided that when the side chains of the following pairs of amino acid residues,  $A_{13}$  and  $A_{17}$  or  $A_{26}$  and  $A_{30}$  are linked through an amide, disulfide or lanthionine bond to form a bridge, then the side chains of at least one of the following pairs of amino acid residues,  $A_{10}$

30

and A<sub>14</sub>, A<sub>14</sub> and A<sub>18</sub>, A<sub>17</sub> and A<sub>21</sub>, A<sub>18</sub> and A<sub>22</sub>, A<sub>21</sub> and A<sub>25</sub> and A<sub>25</sub> and A<sub>29</sub> are also linked through an amide, ester, disulfide or lanthionine bond.

3. A method according to Claim 1 for preparing

5

cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)[Ala<sup>1</sup>,Nle<sup>8</sup>,Lys<sup>18</sup>,Asp<sup>22</sup>,Leu<sup>27</sup>]hPTH(1-31)NH<sub>2</sub>

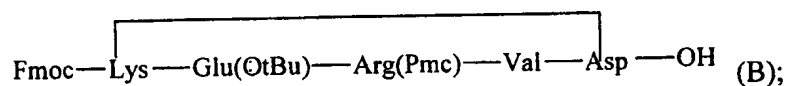
which method comprises the steps of

(a) preparing the resin-bound peptide fragment A:

10

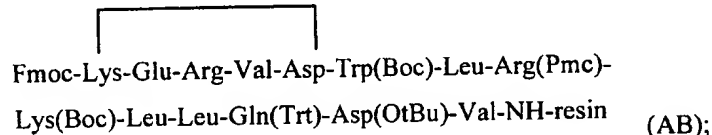
Fmoc-Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin (A);

(b) preparing the cyclic peptide fragment



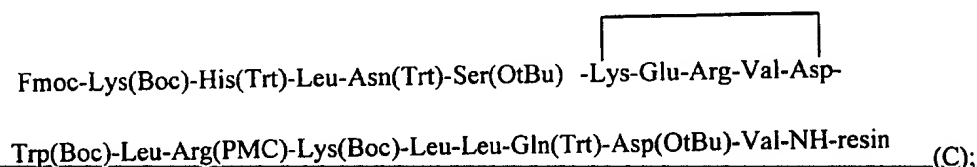
15

(c) coupling peptide fragment A of step (a) with peptide fragment B of step (b) to form intermediate peptide AB



(d) sequentially adding to peptide AB Ser(OtBu), Asn(Trt), Leu, His(Trt), and Lys(Boc)

20 to form intermediate peptide C:



(e) preparing peptide fragment D:

25

Fmoc-Ala-Val-Ser(OtBu)-Glu(OtBu)-Ile-Gln(Trt)-Leu-Nle-His(Trt)-Asn(Trt)-Leu-Gly-OH (D);

(f) coupling fragments C and D to form resin-bound peptide E:

Fmoc-Ala-Val-Ser(OtBu)-Glu(OtBu)-Ile-Gln(Trt)-Leu-Nle-His(Trt)-Asn(Trt)-Leu-Gly-

Lys(Boc)-His(Trt)-Leu-Asn(Trt)-Ser(OtBu) -Lys-Glu-Arg-Val-Asp-

Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin (E);

and

(g) cleaving the resin and deprotecting.

5

4. A peptide suitable as an intermediate for performing the method of Claim 3, selected from the group consisting of:

Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin,

10

Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-OH,

cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)Fmoc-Lys-Glu-Arg-Val-Asp-Trp(Boc)-Leu-Arg(Pmc)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin,

15

Cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)Fmoc-Lys(Boc)-His(Trt)-Leu-Asn(Trt)-Ser(OtBu)-Lys-Glu-Arg-Val-Asp-Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin,

Fmoc-Ala-Val-Ser(OtBu)-Glu(OtBu)-Ile-Gln(Trt)-Leu-Nle-His(Trt)-Asn(Trt)-Leu-Gly-OH,

20

Cyclo(Lys<sup>18</sup>-Asp<sup>22</sup>)Fmoc-Ala-Val-Ser(OtBu)-Glu(OtBu)-Ile-Gln(Trt)-Leu-Nle-His(Trt)-Asn(Trt)-Leu-Gly-  
Lys(Boc)-His(Trt)-Leu-Asn(Trt)-Ser(OtBu)-Lys-Glu-Arg-Val-Asp-  
Trp(Boc)-Leu-Arg(PMC)-Lys(Boc)-Leu-Leu-Gln(Trt)-Asp(OtBu)-Val-NH-resin,

25

BOC-Val-Asp(OAllyl)-Obzl,

- HCl•Val-Asp(OAllyl)-Obzl,
- FMOC-Arg(PMC)-Val-Asp(OAllyl)-Obzl,
- 5 Arg(PMC)-Val-Asp(OAll)-Obzl,
- FMOC-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-Obzl,
- Glu(OtBu)-(L)Arg(PMC)-Val-Asp(OAllyl)-Obzl,
- 10 FMOC-Lys(Alloc)-Glu(OtBu)-Arg(PMC)-Val-Asp(OAllyl)-Obzl,
- FMOC-Lys-Glu(OtBu)-Arg(PMC)-Val-Asp-Obzl,
- 15 FMOC-cyclo(Lys-Asp)Lys-Glu-Arg-Val-Asp-Obzl,
- FMOC-cyclo(Lys-Asp)Lys-Glu-Arg-Val-Asp-OH,
- Fmoc-Lys(Alloc)-Glu(OtBu)-Arg(Pmc)-Val-Asp(OAllyl)-Chlorotrityl resin,
- 20 Fmoc-Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-Chlorotrityl resin, and
- Fmoc-cyclo(Lys-Asp)Lys-Glu(OtBu)-Arg(Pmc)-Val-Asp-Chlorotrityl resin.
-

1  
SEQUENCE LISTING

<110> Sledeski, Adam W.  
Mencel, James J.

<120> PROCESS FOR THE PREPARATION OF RESIN-BOUND CYCLIC  
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<160> 47

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<223> Nle

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<222> (31)

<223> "This C-terminal amino acid is an amide, i.e.,  
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Ala Val Ser Glu Ile Gln Leu Xaa His Asn Leu Gly Lys His Leu Asn  
1 5 10 15

Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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<220>

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<222> (31)

<223> "This C-terminal amino acid is an amide, i.e., CONH2."

<400> 2

Ala	Ala	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Lys	His	Leu	Asn
1				5					10					15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
			20					25					30	

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<220>

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<400> 3

Ala	Val	Ala	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Lys	His	Leu	Asn
1				5					10					15	

---

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
			20					25					30	

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<400> 4

Ala Val Ser Ala Ile Gln Leu Xaa His Asn Leu Gly Lys His Leu Asn  
1 5 10 15

Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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<400> 5

Ala Val Ser Glu Ala Gln Leu Xaa His Asn Leu Gly Lys His Leu Asn  
1 5 10 15

Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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<400> 6  
Ala Val Ser Glu Ile Ala Leu Xaa His Asn Leu Gly Lys His Leu Asn  
1 5 10 15  
Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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<400> 7  
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1 5 10 15  
Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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CONH2."

<400> 8  
Ala Val Ser Glu Ile Gln Leu Ala His Asn Leu Gly Lys His Leu Asn  
1 5 10 15  
Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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CONH2."

<400> 9  
Ala Val Ser Glu Ile Gln Leu Xaa His Ala Leu Gly Lys His Leu Asn  
1 5 10 15  
Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

<210> 10  
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&lt;220&gt;

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&lt;222&gt; (8)

&lt;223&gt; Nle

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&lt;221&gt; PEPTIDE

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&lt;223&gt; "The side chains of Lys at position 18 and Asp at position 22 are linked by an amide bond."

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (31)

&lt;223&gt; "This C-terminal amino acid is an amide, i.e., CONH2."

&lt;400&gt; 10

Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Ala	Gly	Lys	His	Leu	Asn
1				5				10						15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
			20				25						30	

&lt;210&gt; 11

&lt;211&gt; 31

&lt;212&gt; PRT

&lt;213&gt; Unknown

&lt;220&gt;

&lt;221&gt; MOD\_RES

&lt;222&gt; (8)

&lt;223&gt; Nle

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (18)..(22)

&lt;223&gt; "The side chains of Lys at position 18 and Asp at position 22 are linked by an amide bond."

&lt;220&gt;

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&lt;222&gt; (31)

&lt;223&gt; "This C-terminal amino acid is an amide, i.e., CONH2."

&lt;400&gt; -11

Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Ala	Lys	His	Leu	Asn
1				5				10						15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
				20			25						30	

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&lt;212&gt; PRT

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<220>

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Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Ala	His	Leu	Asn
1				5					10					15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
			20					25					30	

<210> 13

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<223> Nle

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<220>

<221> PEPTIDE

<222> (31)

<223> "This C-terminal amino acid is an amide, i.e., CONH2-"

---

<400> 13

Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Lys	Ala	Leu	Asn
1				5					10					15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
				20				25					30	

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CONH2."

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1 5 10 15  
Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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position 22 are linked by an amide bond."

<220>  
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CONH2."

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Ala Val Ser Glu Ile Gln Leu Xaa His Asn Leu Gly Lys His Leu Ala  
1 5 10 15  
Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

<210> 16  
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<220>  
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<223> "The side chain of Lys at position 18 and Asp at position 22 are linked by an amide bond."

<220>  
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1 5 10 15  
Asp Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

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<221> PEPTIDE  
<222> (18)..(22)  
<223> "The side chain of Lys at position 18 and Asp at position 22 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (26)..(30)  
<223> "The side chain of Lys at position 26 and Asp at position 30 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (31)

<223> "This C-terminal amino acid is an amide, i.e.,  
CONH2."

<400> 17

Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Lys	His	Leu	Asn
1				5					10					15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val
			20				25						30	

<210> 18

<211> 34

<212> PRT

<213> Unknown

<220>

<221> MOD\_RES

<222> (8)

<223> Nle

<220>

<221> PEPTIDE

<222> (18)..(22)

<223> "The side chains of Lys at position 18 and Asp at  
position 22 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (34)

<223> "This C-terminal amino acid is an amide, i.e.,  
COHN2."

<400> 18

Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Lys	His	Leu	Asn
1				5					10					15	

Ser	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val	His
				20				25					30		

Asn Phe

---

<210> 19

<211> 34

<212> PRT

<213> Unknown

<220>

<221> MOD\_RES

<222> (8)

<223> Nle

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (13)..(17)

&lt;223&gt; "The side chain of Lys at position 13 and Asp at position 17 are linked by an amide bond."

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (18)..(22)

&lt;223&gt; "The side chain of Lys at position 18 and Asp at position 22 are linked by an amide bond."

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (34)

&lt;223&gt; "This C-terminal amino acid is an amide, i.e., CONH2."

&lt;400&gt; 19

Ala	Val	Ser	Glu	Ile	Gln	Leu	Xaa	His	Asn	Leu	Gly	Lys	His	Leu	Asn
1						5				10				15	

Asp	Lys	Glu	Arg	Val	Asp	Trp	Leu	Arg	Lys	Leu	Leu	Gln	Asp	Val	His
			20					25					30		

Asn Phe

&lt;210&gt; 20

&lt;211&gt; 34

&lt;212&gt; PRT

&lt;213&gt; Unknown

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (18)..(22)

&lt;223&gt; "The side chains of Lys at position 18 and Asp at position 22 are linked by an amide bond."

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (34)

&lt;223&gt; "This C-terminal amino acid is an amide, i.e., CONH2."

&lt;400&gt; 20

Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile	Gln
1					5					10				15	

Asp	Lys	Arg	Arg	Arg	Asp	Phe	Leu	His	His	Leu	Ile	Ala	Glu	Ile	His
							20			25				30	

Thr Ala

&lt;210&gt; 21

<211> 34  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (18)..(22)  
<223> "The side chains of Lys at position 18 and Asp at  
position 22 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (34)  
<223> "This C-terminal amino acid is an amide, i.e.,  
CONH2."

<400> 21  
Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln  
1 5 10 15  
Asp Lys Arg Arg Arg Asp Leu Leu Glu Lys Leu Leu Glu Lys Leu His  
20 25 30  
Thr Ala

<210> 22  
<211> 28  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (12)..(16)  
<223> "The side chains of Lys at position 12 and Asp at  
position 16 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (28)  
<223> "This C-terminal amino acid is an amide, i.e.,  
CONH2."

<220>  
<221> MOD\_RES  
<222> (2)  
<223> Nle

---

<400> 22  
Leu Xaa His Asn Leu Gly Lys His Leu Asn Ser Lys Glu Arg Val Asp  
1 5 10 15  
Trp Leu Arg Lys Leu Leu Gln Asp Val His Asn Phe  
20 25

<210> 23

<211> 28  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (12)..(16)  
<223> "The side chains of Lys at position 12 and Asp at  
position 16 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (28)  
<223> "This C-terminal amino acid is an amide, i.e.,  
CONH2."

<400> 23  
Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Lys Arg Arg Arg Asp  
1 5 10 15

Phe Leu His His Leu Ile Ala Glu Ile His Thr Ala  
20 25

<210> 24  
<211> 28  
<212> PRT  
<213> Unknown

<220>  
<221> MOD\_RES  
<222> (8)  
<223> Nle

<220>  
<221> PEPTIDE  
<222> (7)..(11)  
<223> "The side chains of Lys at position 7 and Asp at  
position 11 are lined by an amide bond."

<220>  
<221> PEPTIDE  
<222> (12)..(16)  
<223> "The side chains of Lys at position 12 and Asp at  
position 16 are linked by amide bonds."

<220>  
<221> PEPTIDE  
<222> (28)  
<223> "This C-terminal amino acid is an amide, i.e.,  
CONH2."

<400> 24  
Leu Xaa His Asn Leu Gly Lys His Leu Asn Asp Lys Glu Arg Val Asp  
1 5 10 15

Trp Leu Arg Lys Leu Leu Gln Asp Val His Asn Phe  
20 25

<210> 25  
 <211> 28  
 <212> PRT  
 <213> Unknown

<220>  
 <221> MOD\_RES  
 <222> (2)  
 <223> Nle

<220>  
 <221> PEPTIDE  
 <222> (12)..(16)  
 <223> "The side chains of Lys at position 12 and Asp at position 16 are linked by an amide bond."

<220>  
 <221> PEPTIDE  
 <222> (20)..(24)  
 <223> "The side chains of Lys at position 20 and Asp at position 24 are linked by an amide bond."

<220>  
 <221> PEPTIDE  
 <222> (28)  
 <223> "This C-terminal amino acid is an amide, i.e., CONH2."

<400> 25  
 Leu Xaa His Asn Leu Gly Lys His Leu Asn Ser Lys Glu Arg Val Asp  
           1                  5                  10                  15

Trp Leu Arg Lys Leu Leu Gln Asp Val His Asn Phe  
                   20                  25

<210> 26  
 <211> 34  
 <212> PRT  
 <213> Unknown

<400> 26  
 Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn  
           1                  5                  10                  15

---

Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His  
                   20                  25                  30

Asn Phe

<210> 27  
 <211> 34  
 <212> PRT  
 <213> Unknown

&lt;400&gt; 27

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln  
1 5 10 15

Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile His  
20 25 30

Thr Ala

&lt;210&gt; 28

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Unknown

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (1)

&lt;223&gt; FMOC-Trp(BOC)

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (3)

&lt;223&gt; Arg(PMC)

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (4)

&lt;223&gt; Lys(BOC)

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (7)

&lt;223&gt; Gln(Trt)

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (8)

&lt;223&gt; Asp(OtBu)

&lt;220&gt;

&lt;221&gt; PEPTIDE

&lt;222&gt; (9)

<223> "This C-terminal amino acid is bonded via an anide  
bond to chlorotrityl resin."

&lt;400&gt; 28

Trp Leu Arg Lys Leu Leu Gln Asp Val  
1 5

&lt;210&gt; 29

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Trp (BOC)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> product=Arg (PMC)

<220>  
<221> PEPTIDE  
<222> (4)  
<223> product=Lys (BOC)

<220>  
<221> PEPTIDE  
<222> (7)  
<223> product=Gln (Trt)

<220>  
<221> PEPTIDE  
<222> (8)  
<223> product=Asp (OtBu)

<220>  
<221> PEPTIDE  
<222> (9)  
<223> Other- "This C-terminal amino acid is bonded via  
an amide bond to Rink Amide MBHA resin."

<400> 29  
Trp Leu Arg Lys Leu Leu Gln Asp Val  
1 5

<210> 30  
<211> 5  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Lys

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<220>  
<221> PEPTIDE  
<222> (1) .. (5)  
<223> "The side chains of Lys at position 1 and Asp at  
position 5 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Glu (OtBu)

<220>

<221> PEPTIDE  
<222> (3)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (1)..(5)  
<223> "The side chains of Lys at position 1 and Asp at  
position 5 are linked by an amide bond."

<400> 30  
Lys Glu Arg Val Asp  
1 5

<210> 31  
<211> 14  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Lys

<220>  
<221> PEPTIDE  
<222> (1)..(5)  
<223> "The side chains of Lys at position 1 and Asp at  
position 5 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Glu(OtBu)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (1)..(5)  
<223> "The side chains of Lys at position 1 and Asp at  
position 5 are linked by an amide bond."

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<220>  
<221> PEPTIDE  
<222> (6)  
<223> Trp(BOC)

<220>  
<221> PEPTIDE  
<222> (8)  
<223> Arg(PMC)

<220>

<221> PEPTIDE  
<222> (9)  
<223> Lys (BOC)

<220>  
<221> PEPTIDE  
<222> (12)  
<223> Gln (Trt)

<220>  
<221> PEPTIDE  
<222> (13)  
<223> Asp (OtBu)

<220>  
<221> PEPTIDE  
<222> (14)  
<223> "This C-terminal amino acid is bonded via an amide  
bond to Rink Amide MBHA resin."

<400> 31  
Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
1 5 10

<210> 32  
<211> 19  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> Fmoc-Lys (BOC)

<220>  
<221> PEPTIDE  
<222> (2)  
<223> His (Trt)

<220>  
<221> PEPTIDE  
<222> (4)  
<223> Asn (Trt)

<220>  
<221> PEPTIDE  
<222> (5)  
<223> Ser (OtBu)

<220>  
<221> PEPTIDE  
<222> (6) .. (10)  
<223> "The side chains of Lys at position 6 and Asp at  
position 10 are linked by an amide bond."

<220>  
<221> PEPTIDE

<222> (7)  
<223> Glu(OtBu)

<220>  
<221> PEPTIDE  
<222> (8)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (6)..(10)  
<223> "The side chains of Lys at position 6 and Asp at position 10 are linked by an amide bond."

<220>  
<221> PEPTIDE  
<222> (11)  
<223> Trp(BOC)

<220>  
<221> PEPTIDE  
<222> (13)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (14)  
<223> Lys(BOC)

<220>  
<221> PEPTIDE  
<222> (17)  
<223> Gln(Trt)

<220>  
<221> PEPTIDE  
<222> (18)  
<223> Asp(OtBu)

<220>  
<221> PEPTIDE  
<222> (19)  
<223> "This C-terminal amino acid is bonded via an amide bond to chlorotrityl resin."

<400> 32  
Lys His Leu Asn Ser Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu  
1 5 10 15

Gln Asp Val

<210> 33  
<211> 11  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Ala

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Ser (OtBu)

<220>  
<221> PEPTIDE  
<222> (4)  
<223> Glu (OtBu)

<220>  
<221> PEPTIDE  
<222> (6)  
<223> Gln (Trt)

<220>  
<221> PEPTIDE  
<222> (9)  
<223> His (Trt)

<220>  
<221> PEPTIDE  
<222> (10)  
<223> Asn (Trt)

<400> 33  
Ala Val Ser Glu Ile Gln Leu His Asn Leu Gly  
1 5 10

<210> 34  
<211> 30  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Ala

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Ser (OtBu)

<220>  
<221> PEPTIDE  
<222> (4)  
<223> Glu (OtBu)

<220>  
<221> PEPTIDE  
<222> (6)

<223> Gln(Trt)

<220>

<221> PEPTIDE

<222> (9)

<223> His(Trt)

<220>

<221> PEPTIDE

<222> (10)

<223> Asn(Trt)

<220>

<221> PEPTIDE

<222> (13)

<223> Lys(BOC)

<220>

<221> PEPTIDE

<222> (14)

<223> His(Trt)

<220>

<221> PEPTIDE

<222> (16)

<223> Asn(Trt)

<220>

<221> PEPTIDE

<222> (17)

<223> Ser(OtBu)

<220>

<221> PEPTIDE

<222> (18)..(22)

<223> "The side chains of Lys at position 18 and Asp at position 22 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (19)

<223> Glu(PtBu)

<220>

<221> PEPTIDE

<222> (20)

<223> Arg(PMC)

---

<220>

<221> PEPTIDE

<222> (22)..(30)

<223> "The side chains of Lys at position 13 and Asp at position 30 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (23)

<223> Trp(BOC)

<220>  
<221> PEPTIDE  
<222> (25)  
<223> Arg (PMC)

<220>  
<221> PEPTIDE  
<222> (26)  
<223> Lys (BOC)

<220>  
<221> PEPTIDE  
<222> (29)  
<223> Gln (Trt)

<220>  
<221> PEPTIDE  
<222> (18)  
<223> Asp (OtBu)

<220>  
<221> PEPTIDE  
<222> (30)  
<223> "This C-terminal amino acid is bonded via an amide  
bond to Rink Amide MBHA resin."

<400> 34  
Ala Val Ser Glu Ile Gln Leu His Asn Leu Gly Lys His Leu Asn Ser  
1 5 10 15  
Lys Glu Arg Val Asp Trp Leu Arg Lys Leu Leu Gln Asp Val  
20 25 30

<210> 35  
<211> 2  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Asp (OAllyl)

<220>  
<221> PEPTIDE  
<222> (1)  
<223> BOC-Val

<220>  
<221> PEPTIDE  
<222> (2)  
<223> "This C-terminal amino acid is in the form of an  
ester with benzyl alcohol."

<400> 35  
Val Asp

1

<210> 36  
<211> 2  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> "The N-terminus is in the form of a hydrochloride  
salt."

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Asp(OAllyl)

<220>  
<221> PEPTIDE  
<222> (2)  
<223> "This C-terminal acid is in the form of an ester  
with benzyl alcohol."

<400> 36  
Val Asp  
1

<210> 37  
<211> 3  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Asp(OAllyl)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> "This C-terminal amino acid is in the form of an  
ester with benzyl alcohol."

<400> 37  
Arg Val Asp  
1

<210> 38  
<211> 3

<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Asp(OAllyl)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> "This C-terminal amino acid is in the form of an  
ester with benzyl alcohol."

<400> 38  
Arg Val Asp  
1

<210> 39  
<211> 4  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> Fmoc-Glu(OtBu)

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (4)  
<223> Asp(OAllyl)

<220>  
<221> PEPTIDE  
<222> (4)

<223> "This C-terminal amino acid is in the form of an  
ester with benzyl alcohol."

<400> 39  
Glu Arg Val Asp  
1

<210> 40  
<211> 4  
<212> PRT

<213> Unknown

<220>

<221> PEPTIDE

<222> (1)

<223> Glu(OtBu)

<220>

<221> PEPTIDE

<222> (2)

<223> Arg(PMC)

<220>

<221> PEPTIDE

<222> (4)

<223> Asp(Oallyl)

<220>

<221> PEPTIDE

<222> (4)

<223> "This C-terminal amino acid is in the form of an ester with benzyl alcohol."

<400> 40

Glu Arg Val Asp

1

<210> 41

<211> 5

<212> PRT

<213> Unknown

<220>

<221> PEPTIDE

<222> (1)

<223> FMOC-Lys(Alloc)

<220>

<221> PEPTIDE

<222> (2)

<223> Glu(OtBu)

<220>

<221> PEPTIDE

<222> (3)

<223> Arg(PMC)

<220>

<221> PEPTIDE

<222> (5)

<223> Asp(Oallyl)

<220>

<221> PEPTIDE

<222> (5)

<223> "This C-terminal amino acid is in the form of an ester with benzyl alcohol."

<400> 41

Lys Glu Arg Val Asp

1 5

<210> 42

<211> 5

<212> PRT

<213> Unknown

<220>

<221> PEPTIDE

<222> (1)

<223> FMOC-Lys

<220>

<221> PEPTIDE

<222> (2)

<223> Glu(OtBu)

<220>

<221> PEPTIDE

<222> (3)

<223> Arg (PMC)

<220>

<221> PEPTIDE

<222> (5)

<223> "This C-terminal amino acid is in the form of an ester with benzyl alcohol."

<400> 42

Lys Glu Arg Val Asp

1 5

<210> 43

<211> 5

<212> PRT

<213> Unknown

<220>

<221> PEPTIDE

<222> (1)

<223> FMOC-Lys

---

<220>

<221> PEPTIDE

<222> (1)..(5)

<223> "The side chains of Lys at position 1 and Asp at position 5 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (2)

<223> Glu(OtBu)

<220>

<221> PEPTIDE

<222> (3)

<223> Arg(PMC)

<220>

<221> PEPTIDE

<222> (5)

<223> "The side chains of Lys at position 1 and Asp and position 5 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (5)

<223> "This C-terminal amino acid is in the form of an ester with benzyl alcohol."

<400> 43

Lys Glu Arg Val Asp

1

5

<210> 44

<211> 5

<212> PRT

<213> Unknown

<220>

<221> PEPTIDE

<222> (1)

<223> Fmoc-Lys

<220>

<221> PEPTIDE

<222> (1) .. (5)

<223> "The side chains of Lys at position 1 and Asp at position 5 are linked by an amide bond."

<220>

<221> PEPTIDE

<222> (2)

<223> Glu(OtBu)

<220>

<221> PEPTIDE

<222> (3)

<223> Arg(PMC)

---

<220>

<221> PEPTIDE

<222> (5)

<223> "The side chains of Lys at position 1 and Asp at position 5 are linked by an amide bond."

<400> 44

Lys Glu Arg Val Asp

1

5

<210> 45  
<211> 5  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Lys(Alloc)

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Glu(OtBu)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Arg(PMC)

<220>  
<221> PEPTIDE  
<222> (5)  
<223> Asp(Oallyl)

<220>  
<221> PEPTIDE  
<222> (5)  
<223> "The C-terminus Asp is bonded via an amide to  
chlorotrityl resin."

<400> 45  
Lys Glu Arg Val Asp  
1 5

<210> 46  
<211> 5  
<212> PRT  
<213> Unknown

<220>  
<221> PEPTIDE  
<222> (1)  
<223> FMOC-Lys

---

<220>  
<221> PEPTIDE  
<222> (2)  
<223> Glu(OtBu)

<220>  
<221> PEPTIDE  
<222> (3)  
<223> Arg(PMC)

<220>

<221> PEPTIDE  
<222> (5)  
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resin."

<400> 47  
Lys Glu Arg Val Asp  
1 5

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/08435

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :C07K 1/04

US CL :530/334

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/334

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS CAS Online

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,674,839 A (HRUBY et al) 07 October 1997, see entire document.	1-4
A	US 5,569,742 A (KIRBY et al) 29 October 1996, col 6, line 64 to col 7, line 11.	1-4
A	US 5,508,382 A (OHSAKI et al) 16 April 1996, see entire document.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	
*B* earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	*A* document member of the same patent family

Date of the actual completion of the international search

05 AUGUST 1999

Date of mailing of the international search report

30 AUG 1999

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